Nutraceutical studies of *Solanum torvum* Swartz.

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The present study deals with the taxonomic details, geographical distribution and nutritional aspects of *Solanum torvum* Swartz. used as a vegetable in the states of North East India as well as in several South Indian states. It is also widely distributed in Uttarakhand, though its edible uses have not been reported from this small state in North India. We conducted a study on the nutritional potential of *S. torvum* berries by evaluating fat, carbohydrates, proteins, energy, total ash and water content using standard methods. HPLC profiling, as well as HPTLC were performed using an in-house protocol developed at Patanjali Research Foundation, Haridwar, Uttarakhand. The presence of triterpenoids, glycosides, alkaloids, flavonoids, phenols, saponins and tannins indicates the nutritional and medicinal importance of *S. torvum*. Biochemical compounds like carbohydrates (15.5% w/w), proteins (0.31%), fats (1.09% w/w), total ash (1.03% w/w) and water content (82.41%) represent its appreciable nutritional value, especially as a low-fat diet and also its contribution to fighting against nutrient deficiencies.

Keywords: Biochemical composition, geographical distribution, nutritional value, *Solanum torvum*, taxonomic study.

THE genus *Solanum* L. in India is represented by 45 species domesticated, usually for their fruits and leaves, eaten raw and cooked; some are even used for medicinal purposes. These species are popularly well known in African countries as a diet and traditional medicine, but in India, the dietary importance of most of these species is not well known, especially in the state of Uttarakhand. A recent review of the *Solanum* L. species suggests they may be a good source of different phytochemicals, especially phenols and flavonoids with high antioxidant activities[1–4]. As the nutritional attributes of *Solanum torvum* from Uttarakhand are not known so far, the present study aimed to evaluate the phytoconstituents as well as biochemical compounds in the berries so that they can be recommended in the Indian diet. The main phenolic compounds from these berries were found to be highest when compared with those of other species of the genus, i.e. *Solanum aethiopicum* and *Solanum macrocarpon*[5].

The berries contain minerals like iron, manganese, calcium, copper, zinc and vitamins A and C[6]. They are eaten as vegetables and used in the treatment of enlarged spleen. They also burned, and the fumes were inhaled to get relief from toothache[7]. In Ghana, berry juice is used to treat anaemia and other ailments[8].

The green fresh fruits are used as an ingredient in certain Thai curries or in raw form in Thai chilli paste. In Laos and Jamaican cuisine, the fruits are used to prepare curries[9], in soups and sauces in Côte d'Ivoire[10] and as herbal tea[11]. It is thus one of the common wild plants known to improve nutrition. In South India, the leaves are utilized as leafy vegetables, while the fruits are eaten cooked or raw[12]. In the states of North East India, particularly Assam and Arunachal Pradesh, the berries are eaten as vegetables[8,13,14].

The present study shows the presence of steroids, alkaloids, flavonoids, phenolics, saponins and tannins in the berries, which are well acknowledged for their health-promoting activities and also helpful in maintaining metabolic functions in the body[15]. It has been reported that the phenolic compounds extracted from different parts of *S. torvum* exhibit antioxidant activity[16]. The plants also have antimycobacterial and cytotoxic activities[17].

Synonyms: *Solanum ferrugineum* Jacq., *Solanum mayanum* Lundell, *Solanum verapazense* Standl. & Steyerm.

During various exploration studies along with global positioning in the states of Arunachal Pradesh (27°44 N/ 99.94°33.203 E), Assam (26°43.814 N/92°58.687 E), Manipur (25°16.898 N/94°.09.978 E), Meghalaya (25°19.249 N/ 92°21.916 E), and Mizoram (24°13.363 N/92°40.399 E) of Northeast India. The market surveys of the vegetable shops of *S. torvvum* were recorded and the herbarium vouchers were identified by matching the specimens kept in the herbarium and deposited in the herbarium of Regional Ayurveda Research Institute Itanagar (ARRI). Further, during the exploration in Dehradun (30°30.078 N, 78°20.7469 E) and Haridwar (29°54′300 N, 78°00′780 E) of Uttarakhand the plant samples collected from the selected areas were also identified by matching the specimens with the authenticated herbarium in the herbarium and deposited in the Herbarium of Patanjali Research Foundation, Haridwar (PRFH). The sample vouchers were prepared by poisoning with 5% mercuric chloride solution in ethanol and mounting on herbarium sheets[18], and deposited in the respective herbaria. Proximate analysis and phytochemical screening were performed at the Department of Chemistry, Patanjali Research Foundation, Haridwar using solvents and chemicals of analytical and laboratory grade. All samples were analysed in triplicate.

The samples were prepared by crushing the berries (250 g) to make a paste stored in an airtight container, well-labelled and kept in a cool, dry place for phytochemical screening.

For proximate analysis, the nutrient contents were determined using standard protocols; protein content, fat (IP: VoI I); moisture, carbohydrate, energy (IS: 7219, 1656

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and 14433 respectively); total ash (AOAC: 922.06). The Kjeldahl method [IS 7219(1973)] was used for protein determination. For this, 0.2 g of sample was taken in a digestion flask, to which was added 0.7 g mercury oxide, 15 g powdered potassium sulphate and 25 ml sulphuric acid. A small amount of paraffin was also added to reduce foaming. The sample was digested using a digestion burner until the solution become clear. It was continuously boiled for 1–2 h. To the digested sample was added 200 ml distilled water followed by 25 ml thiosulphate to precipitate mercury. A few zinc granules were added to prevent bumping, and 25 ml of sodium hydroxide was added to make an alkaline solution. The flask was immediately connected to a distillation bulb with the tip of the condenser immersed in a measured quantity of standard acid (50 ml, 0.5 N HCl) in the receiver. The flask was rotated to mix the contents thoroughly and heated immediately until all the ammonia was distilled. Then, the receiver was lowered before stopping distillation, and the tip of the condenser was washed with distilled water. Excess acid was back-titrated with standard 0.1 N NaOH solution using methyl red as an indicator.

The process was repeated in triplicate, and the results were expressed as: % Crude protein = 6.25 × % nitrogen (% nitrogen = $(S - B) \times N \times 0.014 \times D$/weight of the sample × V × 100), where B is the blank titration reading, D the dilution of sample after digestion, N the normality of HCl, S the sample titration reading, V the volume taken for distillation and 0.014 is the milli equivalent weight of nitrogen.

Fat determination was carried out with 1 g of the sample passed through 1 mm sieve and saturated with 1 ml of ethanol. Next, 5 ml HCl was added. The sample was kept in a preheated water bath (75.5°C) for 40 min and shaken occasionally. It was then removed and allowed to cool at room temperature. Next, 5 ml of ethanol was added to it, followed by 24 ml anhydrous ether in two steps with orbital shaking such that the ether and the residue separate. Collect the top layer and filter it by using filter paper into a previously weighed beaker (150 ml), repeat the same procedure for three times with the remaining material. The ether and water contained in the beaker were evaporated and thereafter kept in an oven at 135°C for 10 min so that it is completely dried and the weight is recorded to be completely constant weight (±0.01 g), percentage of dry matter (percentage crude fat = weight of fat/weight of sample × 100).

Total ash was determined using 4 g of air-dried sample in a previously weighed crucible incinerated gently to a temperature of 675°C for 2 h. The results were expressed as:

Percentage ash = Difference in weight of ash/ weight of sample × 100.

Similarly, moisture content was estimated by taking 5 g of fresh sample, which was transferred to a previously dried and weighed crucible. It was then placed in an oven for 5 h at 105°C. After that cool the dish and weight, repeat the same process at half an hour interval until the difference between two successive weight correspondence to, not more than 0.25 per cent. Record the obtain weight and calculate the moisture content.

Percentage moisture = Weight of wet sample – weight of dry sample/weight of wet sample × 100.

The carbohydrate content was determined by taking the difference between 100 and the sum of moisture, protein, fat and ash contents in the sample. The energy content of the berries was calculated from the following formula:

Total energy (per cent by mass) = $9 \times A + 4\,(B + C)$,

where A is the per cent by mass of fat, B the per cent by mass of total proteins and C is the per cent by mass of carbohydrates.

The phytoconstituents, such as triterpenoids, glycosides, saponins and tannins, were determined using the reference method[19]. For this, 0.5 g of sample was mixed with 2 ml of chloroform. Then, 2 ml each of concentrated sulphuric acid and acetic acid was added to the mixture. The appearance of a greenish colour confirmed the presence of steroids. Next, 2 ml hydrochloric acid was added. The appearance of brown colour confirmed the presence of glycosides. Similarly, 0.5 g of sample was mixed in a test tube with 5 ml of distilled water and shaken vigorously. The frothing that persisted on warming indicated the presence of saponin. However, the appearance of dark blue colour with $FeCl_3$ solution showed the presence of tannin. The alkaloids, phenolic compounds and anthocyanin were determined by the reference method[20]. To 0.5 g of sample, 2 ml of Dragendroff reagent was added. The appearance of a reddish-brown precipitate confirmed the presence of alkaloids. However, when 2 ml of 2 N hydrochloric acid was added to 0.5 g of crushed sample, no pink–red colour appeared, and even after further dropwise addition of ammonia to the mixture, no purple–blue colour appeared, this indicated the absence of anthocyanin. The presence of phenolic compound was detected by a simple qualitative test for the phenol group using 2% ferric chloride solution in 0.5 g of sample mixed with 2 ml of distilled water. The formation of the blue or green colour showed the presence of a phenol group, indicating the presence of phenolic compounds; however, a detailed study of compounds is the need of the hour. The flavonoids were determined using the reference method[21]. To 0.5 g of the sample, a few drops of NaOH solution were added. A yellow-coloured solution was formed, which disappeared with the addition of dilute hydrochloric acid, indicating the presence of flavonoids. For the determination of gums and mucilage, 1 g of sample was mixed with 10 ml distilled water. To the mixture, when 25 ml of absolute alcohol was added with constant stirring, no white cloudy precipitate appeared, indicating the absence of gums and mucilage[22].

For HPLC profiling, 1 g of sample paste was dissolved in 5 ml hydromethanol (80 methanol : 20 water), sonicated

for 20 min, and the clear supernatant was used for analysis. The mobile phase used was (A) 0.1% acetic acid in water and (B) acetonitrile (HPLC-grade). The microlitre of the clear sample was injected, with the flow rate of 1 ml/min, the sample is then pass through column (350 C; Shodex, C18 (4.6 × 250 mm, 5 μl)) and on the basis of affinity with solvent system the compounds are eluted and chromatogram is obtained at 254 nm. To separate polar, mid-polar and non-polar compounds, the composition of the mobile phase was gradually changed from 0% to 95% and then brought back to its initial composition in 80 min. The samples were taken in duplicate.

For HPTLC fingerprinting, 1 g of the sample was dissolved in 5 ml methanol, sonicated for 20 min and centrifuged. The clear supernatant was used for analysis. The mobile phase used was: chloroform : ethyl acetate : formic acid (4.5 : 4.0 : 1.5 v/v/v) (A) under 366 nm and (B) under white light after derivatization by anisaldehydes.

S. torvum is one of the common wild plants known to improve nutrition. It is a 3 m tall shrub with few branches; stem with straight prickles; leaves broadly ovate, shallow cordate, acuminate, densely stellate tomentose beneath and less so above, sparsely prickly on the petiole and the lower surface of the midrib. Inflorescence axillary corymbose cyme with many flowers. Flowers white, calyx unarmed, apiculate, corolla pubescent with stellate hairs, outside, lobes spreading, berries globose, green 1–1.2 cm; calyx persistent (Figure 1).

The plant is native to Mexico, North and South America, Eastern Brazil and the Caribbean; however, it is also found in other parts of the world. In India, it is found mainly in the tropical regions, including the Himalayan states (25°16.898N, 94°09′978E (Assam), 25°48.941N, 94°08′410E (Assam); 25°48′614N, 93°47′278E (Nagaland); 25°16.898N, 94°09′978E (Manipur); 29°54′300N, 78°00′780E (Haridwar); 29°54′43N, 78°126E (Haridwar); 29°54′310N, 78°03′400E (Haridwar); 29°90′7445N, 78°00′2829E (Haridwar); 30°26.9006N, 78°4.3072E (Dehradun); 30030.078N, 78020.7469E (Dehradun)), except the western desert area. It is commonly known as pea eggplant, plate brush, Turkey berry, devil's fig, etc. In India, it is called hathibhekuri in Asamese, titbaigun in Bengali and Sundai in Tamil. The berries of *S. torvum* are a viable source of nutrients and phytoconstituents. Phytochemical screening of the berries showed the presence of steroids, glycosides, alkaloids, flavonoids, phenols, and saponins. However, anthocyanin, gums and mucilage were absent (Table 1 and Figure 2). The berries contain 71.69 kcal/100 g energy, protein and fat content of 0.31% w/w and 1.09% respectively in the fresh seeds, with high water content (Table 2). The present study shows the presence of steroids, alkaloids, flavonoids, phenolics, saponins and tannins well acknowledged for their health-promoting activities and helpful in maintaining metabolic functions in the body[14]. In a comparative study, it was found that flavonoids and terpenoids/steroids were present in both the fresh and boiled leaves of *S. torvum*, but were absent in the fresh and boiled fruits. However, no significant difference was observed in the minerals (iron, calcium and potassium) and vitamins (C, A and lutein) present in fresh and dried berries, indicating that the dried berries are also a good source of nutrients and can be utilized after proper storing[23,24]. Saponins help reduce the risk of cancer and blood lipid accumulation. They also help improve the immune system and prevent future disease conditions. Not only *S. torvum* but other species of the genus *Solanum* also contain these phytochemicals in both berries and leaves. In an analysis of the nutritive values of different *Solanum* species, the quantity of carbohydrates, proteins, vitamins C, D, E, and almost all minerals was highest in *S. torvum.* However, *S. melongena* var. *insanum* had the highest amount of PUFA (poly unsaturated fatty acid) and MUFA (mono unsaturated fatty acid). The dietary

Figure 1. Photographs showing habit of *Solanum torvum* Sw. in Haridwar with its GPS location.

Table 1. Phytochemical screening

Chemical group	Present/absent
Steroids (Triterpenoids)	Present
Glycosides	Present
Alkaloids	Present
Flavonoids	Present
Phenols	Present
Saponin	Present
Tannin	Present
Gums and mucilage	Absent
Anthocyanin	Absent

Table 2. Nutritional analysis

Parameter	Results (unit)
Protein	0.31% w/w
Fat	1.09% w/w
Total ash	1.03% w/w
Water determination	82.41% w/w
Carbohydrate	15.16% w/w
Energy	71.69 kcal/100 g

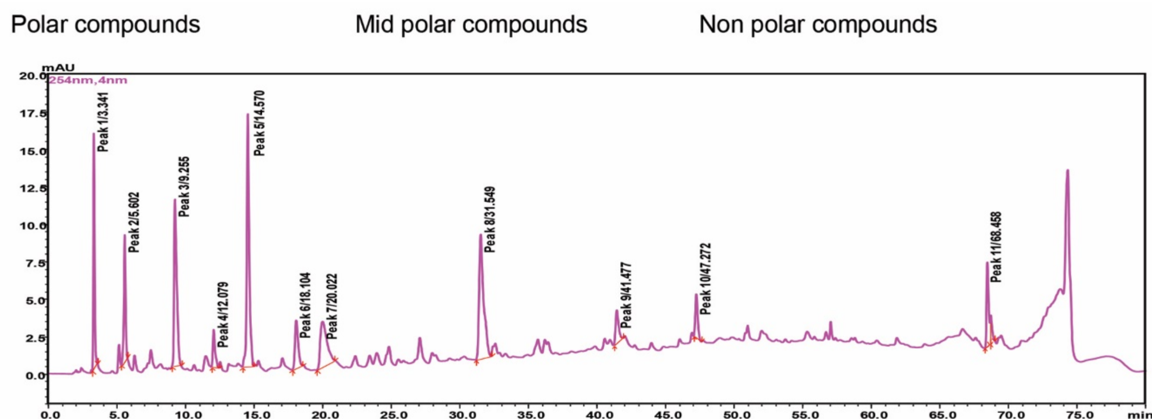

Figure 2. Chromatogram of *S. torvum* at 254 nm by HPLC.

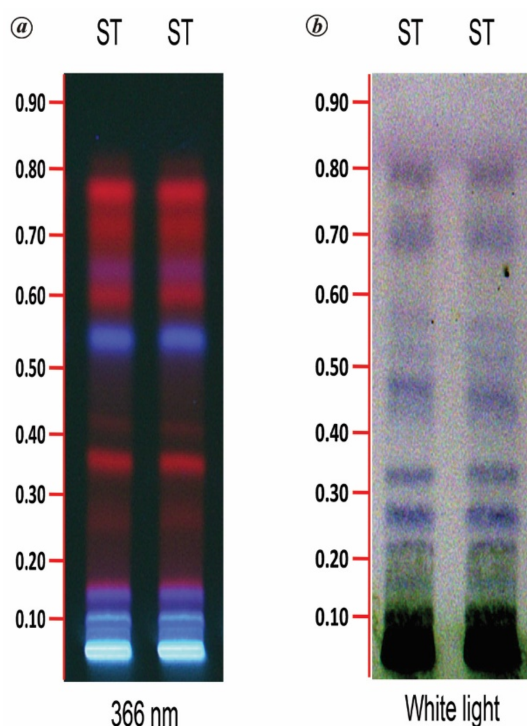

Figure 3. HPTLC fingerprinting of *S. torvum* at 366 nm and white light.

Table 3. The value of peaks at 254 nm

Sl. no.	RT	Area	Area (%)
Peak 1	3.341	96,644	9.523
Peak 2	5.602	70,177	6.915
Peak 3	9.255	146,433	14.429
Peak 4	12.079	28,360	2.795
Peak 5	14.570	213,129	21.001
Peak 6	18.104	50,759	5.002
Peak 7	20.022	98,990	9.754
Peak 8	31.549	186,534	18.381
Peak 9	41.477	31,621	3.116
Peak 10	47.272	36,148	3.562
Peak 11	68.458	56,043	5.522

fibres were highest in *S. melongena* along with vitamins A, K, B_1 and B_2 (ref. 23). Nutrient analysis of a few wild edible fruits from the deciduous forest zone of India revealed that the maximum amount of vitamin C/ascorbic acid in the berries of *S. torvum* (37.4 mg/100 g), compared to pomegranate, papaya, strawberry, guava, banana, mango and sapota[25]. The low-fat content of berries (1.09% w/w) and high water content (82.41% w/w) suggest that they can be consumed as an anti-hypertensive diet and by obese people. Other studies also indicated 80.5% water content in the berries[26]. The polar and non-polar compounds were detected by HPLC profiling. For HPLC analysis C18 column (4.6 × 250 mm, 5 μl) was used with flow rate of 1 ml/min, the phyto-metabolites were detected at 254 nm. Symmetrical, sharp and well-resolved peaks were observed, indicating polar compounds (Figure 2 and Table 3). HPLC profiling showed that peaks 1–7 were polar compounds that eluted first, while peaks 8–10 were mid-polar, and peak 11 was non-polar and eluted at the end.

HPTLC fingerprinting showed the presence of red–blue fluorescent bands under UV 366 nm, while blue–purple bands were observed under white light after derivatization (Figure 3). HPTLC fingerprinting also showed six major spots with *Rf* values ranging from 0.27, 0.37, 0.55, 0.61, 0.73 to 0.76 at UV 366 nm, while six major spots with *Rf* values ranging from 0.21, 0.26, 0.33, 0.48, 0.73 to 0.85 were visible under white light after derivatization by anisaldehyde sulphuric acid. The HPLC chromatogram and HPTLC fingerprint shows different phyto-metabolites, this indicate that these berries contain various compounds that could be potential for working in identification and quantification of identified compounds for future prospective, although some compounds has already been documented by this plant.

The present study suggests that *S. torvum* berries can be a good source of antioxidants, useful to improve nutrition, and can thus be used in our diet. Phytochemical and biochemical analyses by other researchers also testify to the biological activities of the berries[7,27,28]. The results of this study

advise people to consume this species of Solanum since it has a low calorie, high antioxidant content diet and can be cooked naturally with basic ingredients and spices.

Conflict of interest: The authors declare that they have no conflict of interest.

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