NUTRACEUTICAL STUDIES OF SOLANUM TORVUM SWARTZ

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The paper deals with the taxonomic details, geographical distribution and nutritional aspects of *Solanum torvum* used as vegetable in states of North-east India as well as in several South-Indian states also widely distributed in the state of Uttarakhand however, its edible uses are not reported from this part being a small state in India. The authors conducted a study on its nutritional potential of berries by evaluating fat, carbohydrates, proteins, energy, total ash and water content by standard methods. However, HPLC profiling as well as HPTLC was performed using an in-house protocol developed at Patanjali Research Institute, Haridwar, Uttarakhand. The presence of triterpenoids, glycosides, alkaloids, flavonoids, phenols, saponins tannins indicates its nutritional and medicinal importance. The 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%) 71.69% represents its appreciable nutritional value specially as low fat diet and also can contribute to the fight against nutrient deficiencies.

Keywords: *Solanum torvum*, Uttarakhand, biochemical composition, nutritional value.

Introduction

The genus *Solanum* L. in India is represented by 45 species which are domesticated usually for their fruits and leaves, eaten both 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 are not very well known especially in the state of Uttarakhand. The recent review of *Solanum* L. species is suggestive that they may be a good source of different phytochemicals specially phenols and flavonoids with high antioxidant activities[1,2,3,4]. As the nutritional attributes of *Solanum torvum* from Uttarakhand have not been known so far, thus it justifies our present study with the main objective to evaluate the phytoconstituents as well as biochemical compounds in the berries so that it can be recommended in the common man's diet here. The main phenolic compound from these berries were found to be highest when compared with that of other species of genus i.e., *S. aethiopicum* and *S. macrocarpon*[5].

The fruits contain minerals like iron, manganese, calcium, copper, zinc, vitamins A and C[6]. Fruit sare eaten as vegetable and are said to be good for the treatment of enlargement of the spleen. These are also burnt and the fumes are inhaled as helps to get relief in toothache[7]. In Ghana berry juice is used for treatment of anemia and other ailments[8].

The green fresh fruits are used, as an ingredient in certain Thai curries or raw in certain Thai chili pastes (Nam phrik). In Laos and Jamaican cuisine, the fruits are used in preparation of curries[9], incorporated in the soups and sauces in the Côte d'Ivoire[10] and as herbal tea[11]. It is thus one of the common wild plants known to be helpful in nutrition. In South India the leaves are utilized as leafy vegetable and fruits are eaten cooked or raw and is regarded as an essential ingredient in the diet[12]. In the North-eastern states of India particularly Assam and Arunachal Pradesh the berries are eaten as vegetable[13,14,15].

The present investigation shows the presence of steroids, alkaloids, flavonoids, phenolics, saponins, tannins in the berries which are well acknowledged for their health promoting activities and also helpful in maintaining the metabolic functions in the body[16]. It has been reported that the phenolic compounds extracted from different parts of *S. torvum* exhibited anti-oxidant activity[18] as well as the plant has anti-mycobacterial and cytotoxic activities[17].

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

Materials and Method

Furnished the distributional studies of plant during various explorations by taking its Global positioning at selected places with the help of GPS instrument along with the collection of market samples from various markets in north eastern states (Arunachal Pradesh, Assam, Manipur, Meghalaya, Mizoram and Manipur) where the local people sell the plant as vegetable in the local vegetable shops. The sample vouchers were prepared by poisoning with 5% mercuric chloride solution in ethanol and mounting on Herbarium sheets[19] and deposited in the Herbarium of Regional Ayurveda Research Institute, Itanagar. Identified the plant samples properly with help of standard Herbarium kept in Regional Ayurveda Research Institute, Itanagar, further collected the plant samples from Uttarakhand and deposited the samples in the Herbarium of Patanjali Research Institute, Haridwar (PRFH).

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The fresh berries of *S. torvum* were collected during the month of August 2022 from localities nearby Patanjali Research Institute ($29^0$54'300N $78^0$00'780E (Haridwar) - $30^0$30.078N $78^0$20.7469E (Dehradun) regions Uttarakhand. The proximate analysis and phytochemical screening was performed at the Department of Chemistry, Patanjali Research Institute with the use of solvents and chemicals of the analytical and laboratory grade. All samples were analyzed in triplicate.

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

For the proximate analysis the determination of nutrients contents was made by using the standard protocols; protein content, fat (IP:VoI I); moisture, carbohydrate , energy (IS:7219,1656 and 14433 respectively); total ash (AOAC:922.06). The Kjheldal method [IS 7219(1973)] was used for protein determination taking 0.2 g of sample into digestion flask added with 0.7 g mercury oxide and 15 gm powdered potassium sulphate and 25 ml sulphuric acid.Added a small amount of paraffin to reduce foaming and sample was digested using a digestion burner until solution becomes clear and was continuously boiled for 1-2 hours. The digested sample was added with 200 ml distilled water then 25 ml of thiosulphate to precipitate mercury. Few zinc granules were also added to prevent bumping and thereafter 25 ml of sodium hydroxide to make alkaline solution. The flask was immediately connected with distillation bulb with the tip of 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 ammonia has distilled over. Then the receiver was lowered down before stopping distillation and the tip of condenser was washed with distilled water. The excess of acid was back titrated with standard 0.1N NaOH solution using methyl red as indicator.

This was repeated in triplicate and results were expressed as - % Crude protein = 6.25 × % nitrogen (% Nitrogen = (S-B) × N × 0.014 × D /weight of the sample × V × 100) Where B= Blank titration reading; D=Dilution of sample after digestion; N = Normality of HCl; S= Sample titration reading; V= Volume taken for distillation; 0.014 = Milli equivalent weight of Nitrogen.

The fat determination was carried out with1 gm of the sample passed through 1mm sieve and was saturated with 1ml of ethanol further added with 5 ml HCl. The sample was kept in preheated water bath (75.5^0C) for 40 minutes and shaken occasionally. Then was removed and allowed to cool at room temperature. 5 ml of ethanol was added to it then mixed with 24 ml

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anhydrous ether in two steps with orbital shaking such that ether and residue separates. The top layer was pulled off into a dried and tared 150 mL beaker via a Pasteur pipette pouring through a filter paper in a long stem funnel. This step was repeated three more times with 8 ml ether. The ether and water contained in beaker were evaporated and thereafter kept in oven at 135^0C for 10 minutes. The results were obtained by weighing beaker plus fat to +/-0.01 g and results were reported as percentage of dry matter. (Percentage Crude fat = weight of fat /weight of sample × 100).

The determination of the total ash was performed by using 4 gm of air dried sample in a previously weighed crucible was incinerated gently to a temperature of 675^0C for 2 hours. The results were expressed as-

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

Similarly moisture content was also calculated by taking 5 gm of the fresh sample with the transfer to a previously dried and weighed crucible, then placed into an oven and kept for drying for a period of 5 hours at 105°C and thereafter, after cooling expressed in percentage as-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 the moisture, protein, fat and ash contents in the sample. The energy content of the berries is calculated from the formula-

Total energy (percent by mass) = 9 × A + 4 (B + C), where A=percent by mass of fat, B= percent by mass of total protein, C= Percent by mass of carbohydrate

The phytoconstituents such as triterpenoids, glycosides, saponins, tannins were determined using the reference method[20]. 0.5 gm of sample was mixed with 2 ml of chloroform and then (a) 2 ml each of concentrated sulphuric acid and acetic acid was added to the mixture. The appearance of greenish colour confirmed the presence of steroids (b) added 2 ml hydrochloric acid in it. The formation of brown colour confirmed the presence of glycosides. Similarly 0.5 gm 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, anthocyanin were determined by the reference method[21]. In 0.5 gm of sample, on addition of 2 ml of Dragendroff reagent the presence of reddish- brown precipitate confirmed the presence of alkaloids. However, when in 0.5 gm of crushed sample, 2 ml of 2N hydrochloric acid was added

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and no pink- red colour appeared and even after further dropwise addition of ammonia to the mixture, no purple-blue colour indicated the absence of anthocyanin. The presence of phenolic compound was detected by simple qualitative test for phenol group using 2% ferric chloride solution in about 0.5 gm of sample mixed with 2 ml of distilled water. The formation of blue or green colour showed the presence of phenol group indicating the presence of phenolic compounds, however the detailed investigation of those compounds is need of hour. The flavonoids were determined using reference method[22]. In about 0.5 gm of sample, on addition of few drops of NaOH solution yellow coloured solution was formed which disappeared on addition of dilute hydrochloric acid, indicating the presence of flavonoid. For the determination of gums and mucilage, about 1 gm of sample was mixed with 10 ml distilled water. To the mixture, when added 25 ml of absolute alcohol was added with constant stirring no white cloudy precipitate appeared indicating the absence of gums and mucilage[23].

For HPLC profiling 1 gm of sample paste was dissolved in 5 ml hydro-methanol (80 Methanol: 20 Water), sonicated for 20 min and the clear supernatant was used for the analysis. The mobile phase used was A: 0.1% Acetic acid in water, B: Acetonitrile (HPLC grade). A thermostatically controlled column [350 C; Shodex, C18 (4.6 × 250 mm, 5μ)] with a flow rate of 1ml/min, injection volume 10 μl at a wavelength 254 nm. To separate polar, mid polar and non-polar compounds mobile phase gradually changed the composition from 0% to 95% and again brought back to its initial composition in 80 minutes. The samples were taken in duplicate.

For HPTLC Fingerprinting, 1 gm of sample was dissolved in 5 ml methanol, and was sonicated for 20 minutes and centrifuged; clear supernatant was used for the 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.

Results and Discussion

S. torvum is one of the common wild plants known to be helpful in nutrition. It is a shrub up to 3 m tall 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 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.

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The plant is native to native to Mexico to North South America, Eastern Brazil and Caribbean however also found in other parts of world. In India, it is found mainly in tropical region including Himalayan states [25°16.898 N 94°09'978E (Assam), 25^0 48.941 N, 94^0 08'410 E (Assam); 25^0 48 614 N, 93^0 47'278 E (Nagaland); $25^0$16.898N. $94^0$09'978E (Manipur); $29^0$54'300N $78^0$00'780E (Haridwar); $29^0$54'43 $78^0$126E (Haridwar); $29^0$54'310N. $78^0$03'400E (Haridwar); $29^0$90'7445N $78^0$00'2829E (Haridwar); $30^0$26.9006N $78^0$4.3072E (Dehradun); 30030.078N 78020.7469E (Dehradun)] except the Western desert area. It is commonly known as pea egg plant, plate brush, Turkey berry, Devils fig etc. In India, it is called as Hathibhekuri in Asamese, Titbaigun in Bengali, and Sundai in Tamil. The berries of *S. torvum* represent a viable source for nutrients and phyto-constituents and validate it as a good nutritional source. The phytochemical screening of the berries shows the presence of steroids, glycosides, alkaloids, flavonoid, phenols, saponins, however anthocyanin, gum and the absence of the mucilage. (Table-1, Figure 2). The berries also contain 71.69 kcal/100 gm energy while the protein and fat 0.31 % w/w and 1.09 % respectively, in fresh seed, with high water content as shown in table-2. This study shows the presence of steroids, alkaloids, flavonoids, phenolics, saponins, tannins well acknowledged for their health promoting activities and also helpful in maintaining the metabolic functions in the body[15]. In a comparative study it was found that the 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) contents of fresh and dried berries, indicating that the dried berries are also a good source of nutrients and can be utilized on storing it also[25]. The saponins are known to help reduce the risk of cancer and blood lipid accumulation and would help improve the immune system and prevent future disease conditions. Not only *S. torvum* but also other species of genus *Solanum* contain these phytochemicals in both berries and leaves. In an analysis of nutritive values of different *Solanum* species, the highest quantity of carbohydrate, protein, vitamin C, D, E and almost all minerals were in highest amount in *S. torvum* although *S. melongena* var. *insanum* exhibited highest quantities of PUFA (Poly Unsaturated Fatty Acid) and MUFA (Mono Unsaturated Fatty Acid). The dietary fibers were highest in *S. melongena* along with vitamins A, K, B_1 and B_2[24]. The nutrient analysis of few wild edible fruits of deciduous forest zone of India revealed that the maximum amount of vitamin C / ascorbic acid was found in the berries of *S. torvum* (37.4 mg/100 g), as compared to

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pomegranate, papaya, strawberry, guava, banana, mango, sapota[26]. The low fat content of berries (1.09 % w/w) and high water content (82.41% w/w) suggests that one can consume it as anti-hypertensive diet and by obese people with overweight problems. The other studies are also indicative of 80.5% water content in berries[27]. The polar and non-polar compounds were detected by HPLC profiling. In this study, C18 column with 250 mm length and wavelength 254 nm was used with flow rate of 1 ml/min for chromatogram. Symmetrical, sharp and well-resolved peaks were observed indicating polar compounds (Fig. 2; Table 3). HPLC Profiling showed that the peaks 1, 2, 3, 4, 5, 6 and 7 were polar compounds, hence eluted first while peak 8, 9 and 10 were mid polar and peak 11 was non- polar 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. HPTLC Fingerprinting showed 6 major spots with Rf value ranging from 0.27, 0.37, 0.55, 0.61, 0.73 and 0.76 at UV 366 nm while 6 major spots with Rf value ranging from 0.21, 0.26, 0.33, 0.48, 0.73 and 0.85 are visible under white light after derivatization by Anisaldehyde sulphuric acid. The observed data, interprets that this present HPLC and HPTLC study could be indicative to a potential application for identification and quantization of many polar compound present in these berries irrespective of already documented important phytochemicals.

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Conclusion

Based on our studies it is suggestive that *S. torvum* berries can be a good source of antioxidants, may be useful to improve nutrition, and can be used in meals. In addition, the other different phytochemical and biochemical analysis by other different researchers also testifies their biological activities[7,28,29]. The findings of the present study encourage to eat this species of *Solanum* because it is low calorie rich in antioxidants diet, can be prepared in a natural way of cooking with simple cooking ingredients and spices.

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

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