Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
www.phytojournal.com
JPP 2024; 13(4): 136-141
Received: 18-05-2024
Accepted: 22-06-2024

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The nutraceutical studies of the berries of *Solanum violaceum* Ortega, a traditional vegetable

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DOI: https://doi.org/10.22271/phyto.2024.v13.i4b.15004

Abstract
Solanum violaceum is widely used as vegetable in of North-east India. This paper presents the taxonomic details, geographical distribution and nutritional aspects of berries of this plant. The plant is widely used as vegetable in states of North-east India where in the antioxidant properties were reported. It is also used as traditional medicine in the treatment of Asthma, it is widely distributed in different other tropical part of India. A study was conducted to study the nutritional aspects of berries by using standard methods practiced. An in-house protocol was developed at Patanjali Research Institute, Haridwar, Uttarakhand to conduct HPLC profiling as well as HPTLC.The presence of flavonoids (0.031%) and phenols (0.134%), indicates its nutritional and medicinal importance. The biochemical compounds like carbohydrates (14.30% w/w), proteins (01.53%), fats (1.12% w/w), total ash (05.53% w/w), water content (77.52%) and energy level (73.40 kcal). The results indicate that berries are of low fat but of high nutritional value and thus can contribute to the fight against nutrient deficiencies. The study was performed with an objective of Nutritional composition analysis, qualitative analysis of secondary metabolite, HPTLC fingerprinting, and UHPLC ((Ultra High Performance Liquid Chromatography) profiling berries of *S. violaecum*.

Keywords: Nutraceutical, flavonoids, polyphenols, *Solanum violaceum*

Introduction
Solanum violaceum Ortega belonging to family Solanaceae comprising of about 1, 234 species widely distributed throughout temperate and tropical regions of world. In India, 64 species of *Solanum* have been reported inclusive of cultivars [1]. The species of *Solanum* L. are mainly being domesticated for their leaves as well as fruits, consumed as edible delicacies both raw and in cooked formed; some species are also used for medicinal purposes. The *Solanum* species like *S. violaceum* Ortega*, S. torvum* Swartz*, S. nigrum* L. and *S. tuberosum* L. are used as vegetable as well as medicinally for worm infestation and digestive disorders in North East India [2-6]. The various scientific studies confirm the presence of different phytochemicals specially flavonoids and phenols in berries of *Solanum* species which presents high antioxidant activities. The people of North-eastern states of India like Arunachal Pradesh, Assam, Nagaland, Manipur are well aware of the nutritional aspects of *Solanum violaceum* and consume it frequently still the nutritional aspects of the berries are yet to be studied and the present study was carried out to fulfil this aim. The berries are good source of minerals like Fe, Ca, P, Mg, vitamins like A and C [7] and are widely consumed as vegetable and also regarded as good for the treatment of diabetes and respiratory diseases. Its berries are also used as vegetable in Bangladesh and Sri Lanka [8, 9]. Medicinally it is used in asthma, dry cough, catarrh, colic, flatulence, worms, fever and diabetes [10].

The present investigation shows the presence of phenolics, steroids, flavonoids, alkaloids, tannins and saponins, in the berries. These all compounds are widely acknowledged for their antioxidant activities leading to health promoting activities and thereby helpful in maintaining the metabolic functions in the body [11]. The various phenolic compounds extracted from different parts of *S. violaceum* has been shown to exhibit anti-oxidant activity in various studies conducted so far [12]. The plant also exhibits anti-mycobacterial and cytotoxic activities, however antimicrobial, antioxidant, and anthelmintic activities are known however, anti-inflammatory, analgesic, antipyretic, and other pharmacological studies are yet to be studied [13]. The indigenous Garo people of Bangladesh mix the seed with liquor to increase its intoxication effect [14].

Synonyms: *Solanum anguivi* Hook, *Solanum indicum* var. *parvifolium* Dunal; *Solanum kurzii* Brace ex Prain.

Vernacular names: Vrihati (Sanskrit); Phutki, Tit Begun, Brihati Begun, Baikur (Bengali); Pokhongkhesi (Marma tribe); Titbahal by Garo tribe in Bangladesh [6]. Poison Berry and Indian Night Shade in English; of Turkey berry, Thibatu in Sri Lanka [10].

Materials and Methods

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 NE India (Arunachal Pradesh, Assam, Manipur, Meghalaya, Mizoram and Manipur), where local people sell the plants in local vegetable shops. Used 5% mercuric chloride solution in ethyl alcohol for the poisoning of voucher specimens and then mounted them on herbarium sheets according to the method described in reference [15] and deposited in the Herbarium of the Regional Ayurveda Research Institute, Itanagar. Fresh berries of *S. violaceum* were collected in October, 2022 from local markets of Itanagar.
The proximate analysis and phytochemical screening was conducted in the Chemistry Laboratory of Patanjali Research Institute, Haridwar analytical and laboratory grade solvents and chemicals. 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 analysis.

Proximate Analysis

The nutrient contents were determined using standard protocols [16], protein content, fat (AOAC, 2016, 20th edition, Official Methods of Analyses); moisture, carbohydrate, energy (IS: 7219, 1656 and 14433 respectively); LOD (API, part I, Vol. VIII, 2011); total ash (API, part I, Vol. VIII, 2011

Protein

Protein was estimated by using Kjeldahl method (IS: 7219 (1973). 0.2 g of sample was weighed in kjeldahl flask 25 mL of concentrated sulphuric acid was added into it to dissolve the sample completely. Thereafter 1 g mixture of copper sulphate and potassium sulphate (1:20) were added in the sample. The solution was digested kept on heating mental until the becomes clear. It was allowed to cool and then diluted with distilled water up to 200 ml, and then 50 ml of 40% sodium hydroxide was added. Few glass beads were added in mixture to avoid excessive heat. The flask was connected with distillation unit, immersing the tip of condenser in beaker containing 50 ml of 0.1 N HCl. The flask was heated and then the distillate was collected which was about 150 ml. The back titration was carried out using the excess acid with standard 0.1 n sodium hydroxide solution taking methyl red as indicator. Same procedure was followed for blank. The protein content was determined using formula as:

Nitrogen content (N) in g = [(A-B)-(C-D)] 0.0014

Where, A= Volume of 0.1 N Acid (HCl) measured for distillation in ml (for sample), B= Volume of 0.1 N Alkali (NaOH) used for back-titrating A, C= Volume of 0.1 N Acid (HCl) measured for blank distillation in ml, D = Volume of 0.1 N Alkali (NaOH) used for back-titrating C.

$$\text{Protein, percent by mass} = \frac{N \times 100 \times \text{Conversion factor}}{W}$$

Where, N = Mass of nitrogen content in g of original sample, W= Mass of sample in g, Conversion factor = Conversion factor (as per IS: 7219).

Fat

2.0 g of ground, well-mixed sample was taken and then 2 mL of ethanol was added to it and was shaken to moisten all particles. Add 10 mL hydrochloric acid (add HCl and water in the ratio of 25:11 v/v), mix well. Keep it for 30-40 minutes in water bath at 70-80 °C, shake the mixture occasionally to avoid lump formation. Allow cooling at room temperature and adding 1-2 mL ethanol, transfer it to separating funnel. Add 25 mL petroleum ether and shake vigorously for 1 minute. Allow it to stand till upper liquid becomes practically clear. Collect the upper layer and filter it through funnel consisting of small pledged of cotton packed firmly into 150 mL beaker containing several glass beads. Re-extract the liquid remaining in the separating funnel twice, each with 15 mL of diethyl ether and 15 mL of petroleum ether. Shake for about 1 minute after addition of diethyl ether and petroleum ether. Pour clear ether solution through filter into the same beaker as before, and dry it on water bath, then cool to room temperature. Re-dissolve dried fat residue in 10 mL diethyl ether, filter it through small piece of cotton into a pre-dried (30 minutes at 100 °C) and weighed100 mL beaker containing few glass beads, cooled to room temperature in a desiccators and weighed immediately. The procedure for repeated thrice. Use 10 mL of diethyl ether for rinsing cotton and funnel. Evaporate the collected diethyl ether layer on water bath, dry for 90 min at 80-90 °C, cool to room temperature in a desiccator, and weigh immediately.

$$\text{Fat (\% w/w)} = \frac{(W_2 - W_1) \times 100}{W}$$

Where, W_1= Empty beaker weight, in g, W_2 = Weight of beaker and fat, in g, W= Sample weight, in g

Carbohydrate

The difference between 100 and the sum of the moisture, protein, fat and ash contents in the sample gave the carbohydrate content present in the sample.

Total Energy

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.

Total Ash

2 – 4 gm of sample was taken, and then kept in a pre-dried and weighed crucible. The material was then charred on hot plate after charring is completed the crucible containing sample is placed inside muffle furnaces at 600°C for 3-4 hr. after that crucible is removed from muffle furnace and kept inside desiccators until it is cooled, then weigh the crucible, repeat the same heating and cooling process for half an hour interval until constant weight is obtained. Calculate the results of total ash as follows:

Ash (% w/w) = Difference in weight of Ash/weight of sample × 100.

Loss on drying (LOD) is determined by taking 5-10 g of sample in a pre-dried and weighed Petri-dish, place the weighed sample on dish, then keep it in hot air oven at 105°C for 5 hr., after then revive the petri-dish from oven and after cooling note down the weight. Repeat the same process for 30 min interval, until the constant weight is noted.
Calculate the LOD (% w/w): Weight of sample before drying – weight of sample after drying/ Sample weight × 100.

Phytoconstituent Analysis
Steroids and Glycosides
In a test tube mix 0.5 gm of sample with 2 mL of chloroform and then add 2 mL each of concentrated sulphuric acid and acetic acid to it. The appearance of greenish colour confirms presence of steroids. Similarly, if after addition of 2 mL hydrochloric acid in the same mixture formation of brown colour appears it confirms the presence of glycosides [17].

Saponins and Tannins
In a test tube mix 0.5 gm of sample with 5 mL of distilled water and shake the contents vigorously till frothing appears. Warm the contents, if frothing that persisted on warming indicates he presence of saponin. The addition of $FeCl_3$ to sample mixture before warming if results in appearance of dark blue colour shows the presence of tannin [17].

Alkaloids and Phenolic compounds
In a test tube mix 0.5 gm of sample with 2 ml of Dragendroff reagent. The presence of reddish-brown precipitate confirms presence of alkaloids. In a test tube mix 0.5 gm of sample with 2 mL of distilled water and 2% ferric chloride solution. 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 [18].

Flavonoids
In about 0.5 gm of sample, few drops of NaOH solution was added. The formation of yellow coloured solution disappearing on addition of dilute hydrochloric acid, indicates the presence of flavonoid [19].

Anthocyanin
0.5 gm of crushed sample was mixed with 2 mL of 2N hydrochloric acid, no pink-red colour appeared, and even after further dropwise addition of ammonia to the mixture, no purple-blue colour indicates the absence of anthocyanin [18].

Gums and Mucilage
Mix 1 gm of sample with 10 mL distilled water. Then add 25 mL of absolute alcohol in it with constant stirring. The nonappearance of white cloudy precipitate indicates the absence of gums and mucilage [20].

HPLC (High Performance Liquid Chromatography) profiling
The HPLC profiling was performed by taking clear supernatant prepared from 1g of sample paste dissolved in 5 mL hydro-methanol (80 methanol: 20 water), sonicated for 20 minutes. The mobile phase used was (A) 0.1% acetic acid in water and (B) acetonitrile (HPLC-grade). A thermostatically controlled column (350 C; Shodex, C18 (4.6 × 250 mm, 5 µ) with a flow rate of 1 mL/min, injection volume of 10 µl at a wavelength 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.

HPTLC (High Performance Thin Layer Chromatography) Fingerprinting
1 g of 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 (A): 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
S. violaceum is a very common plant growing in Northeastern states as is very helpful to improve nutritional quality. It is shrub ranging from 0.5-1.5m tall, having many branches, densely pubescent throughout with 5-11-rayed stellate hairs. Stems and branches with pale yellow with recurved prickles 4-10 mm, gray in colour, stellate tomentose. Leaves unequal paired; petiole 1.4-3.9 cm; leaf blade ovate, 5-10.5) × 2-8 cm, tomentose with short-stalked, 5-11.5)-rayed stellate hairs adaxially, with long-stalked hairs abaxially, with straight needle like prickles, base cordate or truncate, margin 5-7-sinuate lobed, apex obtuse or acute. Inflorescences extra-axillary, curved racemes, 2-5.8 cm, poorly branched; peduncle up to 1.5 cm, prickly or not. Pedicel 4-15 mm. Sepals lanceolate, 3-5 mm, unequal, pubescent and prickly likewise pedicel. Corolla blue-purple, sometimes white, rotate, petals 1-1.2 × 1-2.2 cm; ovate or ovate-lanceolate, 5.2-8 × 2.1-4.8 mm, obtuse. Filaments to 1 mm; anthers oblong. Style to 10 mm, stellate pubescent. Fruiting stalks stellate pubescent, prickly, erect or sometimes recurved. Persistent calyx reflexed, stellate pubescent, prickly. Berries shiny orange, globose, to 1.2 cm in diam. Seeds sub-discoid. Flowering and fruiting takes place round the year (Figure-1).

Fig 1: Shoot and berries of *Solanum violaceum*

The plant is distributed and sold in the markets of foot hills of Arunachal Pradesh, Assam, Mizoram and Nagaland. The Global positions of the plants is as follows:
[Itanagar and Pasighat in Arunachal Pradesh: (27o.10.38’ N, 93o63.3’ E; 28° 04.12’ N; 95° 19.48’ E), Kolasibin Mizoram (24° 13. 36’ N; 92° 40.39’ E), Harmutti and Vishwanath Chariyali in Assam (27° 7. 25’ N; 93° 51.28’ E; 26° 44.29’ N, 93° 8.20’ E), Mariyani in Nagaland (26° 39.56’N, 94° 19. 41’ E) and Jiribam in Manipur (24°48.00’ N, 93°07.12’ E)].
The plant is native to Indian Subcontinent to Taiwan and West and South Malesia. In India it is distributed in tropical

regions from Northeast to Kerala and Tamil Nadu in Evergreen, semi-evergreen and dry deciduous forests, also in the plains. It is also distributed in Bangladesh, South-Central China, Southeast China, East Himalaya, Hainan, India, Java, Laos, Myanmar, Pakistan, Philippines, Saudi Arabia, Sri Lanka, Taiwan, Thailand, Vietnam, Yemen; cultivated in Andaman Islands, Comoros, Madagascar, Mauritius, Mozambique, New Caledonia, Nicobar Islands, Rodrigues, Réunion [21].

S. violaceum is one of the common wild plants known to be helpful in nutrition. In Northeastern India the berries are eaten as vegetable in cooked form and is regarded as an essential ingredient in the diet with the concept that it is good for digestion and as worm repellent. The berries of *S. violaceum* represent a viable source for nutrients and phyto-constituents and validates it as a good nutritional source. The phytochemical screening of the berries shows the presence of steroids, glycosides, alkaloids, flavonoid, phenols, saponins and gums however, showed the absence of phytosterol, triterpenoids, anthocyanin the mucilage (Table-1).

Table 1: Phytochemical screening of berries of *S. violaceum*

S. No.	Chemical group	Present/absent
1.	Phytosterol	Absent
2.	Cardiac Glycosides	Present
3.	Alkaloids	Present
4.	Flavonoids	Present
5.	Phenols	Present
6.	Saponin	Present
7.	Tannin	Present
8	Gums and mucilage	Present
9	Anthocyanin	Absent
10	Diterpenoid	Present
11	Triterpenoid	Absent
12	Mucilage	Absent

The berries also contain 73.40 kcal/100 gm energy while the protein and fat 01.12% w/w, protein 01.53% carbohydrate 14.30% and ash 05.53% respectively, in fresh berries, with high water content, i.e., 77.52% (Table-2). The phytochemicals in plants are responsible for the defence mechanism in them and provide some level of protection from invasion of disease, infection as well as predators along with it also impart colour in the plant part.

Table 2: Nutritional Composition of berries of *S. violaceum*

SL No.	Parameters	Quantity	Method
1.	Protein, (% w/w)	01.53	IS:7219
2.	Fat, (% w/w)	01.12	AOAC-4.5.02 (959.02)
3.	Total Ash, (% w/w)	05.53	USP-NF <561>
4.	Moisture	77.52	USP <921>
5.	Carbohydrate, per 100 g	14.30	IS: 1656
6.	Energy, Kcal/100g	73.40	IS: 14433

The total flavonoids and polyphenols in berries as determined using HPTLC were found to be 0.031% (w/w) and 0.134% (w/w) respectively. (Table 3,Figures 2, 3, 4).

Table 3: Total flavonoids and polyphenols in the berries of *S. violaceum*

S. No.	Parameters	Quantity in % w/w
1.	Total Flavonoid, equivalent to Quercetin (% w/w)	0.031
2.	Total Polyphenol, equivalent to Gallic acid (% w/w)	0.134

The UHPLC profile indicates the presence of high-polar compounds in berries (Figure 5). In a study the quantitative estimation of phenolic acids was determined in different solvent extracts of *S. kurzii* and *S. gilo* by reversed-phase HPLC method with diode array detection. The HPLC assay showed a well separation of the compounds and the developed method was sensitive, accurate, linear, meticulous and reproducible. The RP-HPLC results showed presence of different phenolic compounds asgentisic acid, gallic acid, protocatechuic acid, caffeic acid, p-hydroxy benzoic acid, vanillic acid, syringic acid, pcoumaric acid, chlorogenic acid, sinapic acid, ferulic acid and ellagic acid in varying amounts.

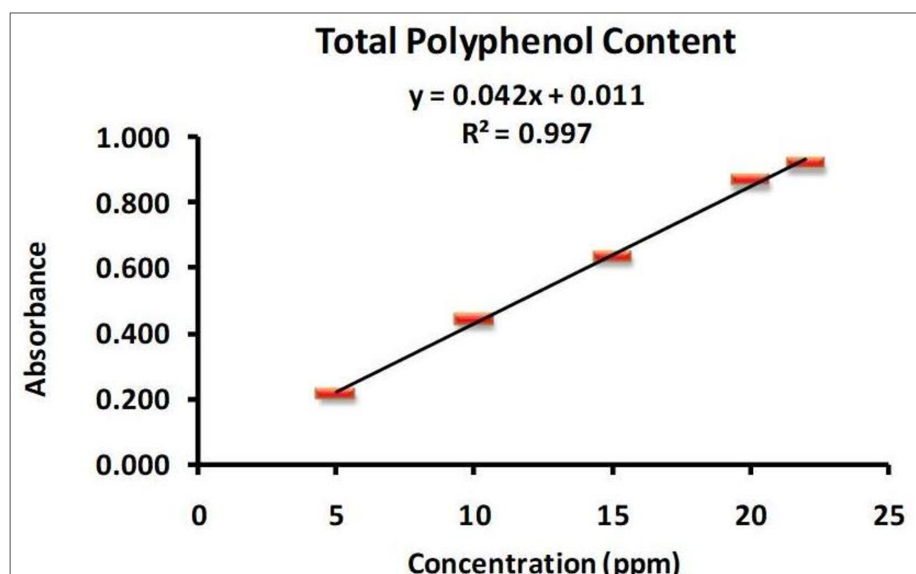

Fig 2: Linearity plot for total polyphenols, gallic acid as a standard

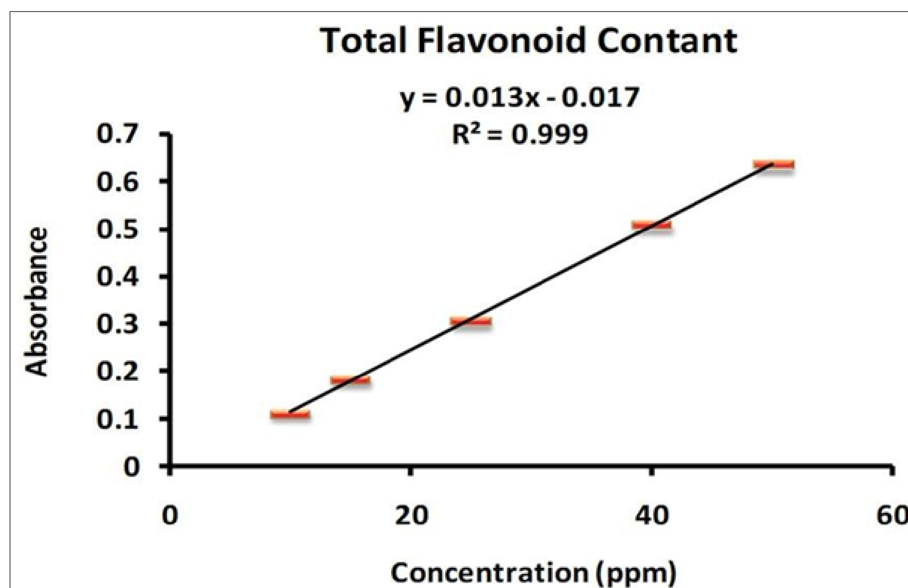

Fig 3: Linearity plot for total flavonoids, quercetin as a standard

Discussion and Conclusion

S. violaceum contains alkaloids, carbohydrates, di-terpenes, flavonoids, glycosides, gums, phenols, proteins, saponins and tannins in the present study as well as previously worked out [12, 13]. In the present investigation total phenolic content of methanolic extract was found to be 54.67 Gallic Acid Equivalent (GAE) per gram of dry extract13. Beries contain 1.8% steroidal alkaloids, while the leaves and roots contain steroidal alkaloids, solanine, solanidine and solasodine [8, 22].

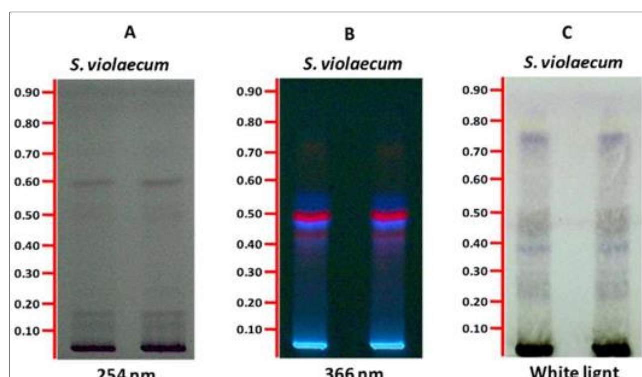

Fig 4: HPTLC fingerprinting of berries of *Solanum violaecum*

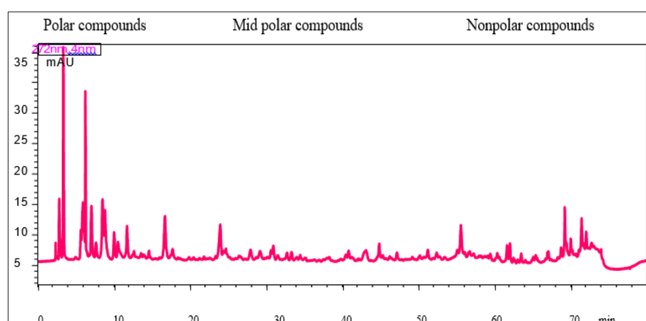

Fig 5: Chromatogram of *Solanum violaecum* by HPTLC fingerprint of berries of at 254 nm.

The present study shows the presence of steroids, alkaloids, flavonoids, phenolics, saponins, tanins well acknowledged for their health promoting activities and also helpful in maintaining the metabolic functions in the body. The quantitative values of Total Flavonoid, equivalent to Quercetin (% w/w) 0.031 and Total Polyphenol, equivalent to Gallic acid (% w/w) 0.134 shows the highly protective values of the berries of *S. violaceum.*

The most widely distributed secondary metabolites in plant are phenolic compounds, which are largely the effect of genotype, environmental conditions and storage method. The various other scientific reports also indicate that the phenolic compounds extracted from different parts of *S. violaceum* exhibited anti-oxidant activity [11] as well as the plant has anti-mycobacterial, anthelmintic, and cytotoxic activities described [13, 16], which support it being the high valued vegetable.

A HPTLC method using photodiode array detector with gradient elution has been developed and validated for the estimation of free phenolic acids (gallic acid, protocatechuic acid, gentisic acid, chlorogenic acid, p-hydroxy benzoic acid, vanillic acid, caffeic acid, syringic acid, pcoumaric acid, ferulic acid, sinapic acid, salicylic acid and ellagic acid), in two different solvent extracts of *Solanum gilo* and *S. kurzii* collected from North-eastern region in India [11].

The present study reveals that eating the berries of *S. violaceum* justified for being good source of antioxidants, and can be included in our diet. Phytochemical and biochemical studies by different other researchers also justifies the use of berries in diet due to its biological activities. Studies encourage eating the berries because of having low calories, antioxidants and easy to domestic preparation by cooking with other vegetables as well as spices.

Acknowledgements

Authors are thankful to Parampujya Swami Ramdev Ji, for providing all the research facilities and encouragements. Thanks are also due to Dr. Anurag Varshney, Vice President, Patanjali Research Foundation and Dr. Jyotish Srivastava, Ex-Head, Chemistry Lab, Patanjali Research Foundation for assistance in conducting the present studies. Supports rendered by the Director General, Central Council for research in Ayurvedic Sciences during the field studies in North-eastern states is also thankfully acknowledged.

Conflict of interest

There is no any kind of conflict of interest amongst the authors.

Author's contribution

Concept and theme of the AB looked the overall guidance, RS developed concept and theme after conducting field studies and compiled the manuscript, RAJ, MJ conducted biochemical analysis, methodology of the paper, UBP prepared the technical analysis, and AS & VPA supervised and draft setting.

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