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FRUITS OF *SOLANUM ACULEATISSIMUM* JACQ.A NUTRACEUTICAL ENRICHED INVASIVEWEED?

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ABSTRACT

Solanum, the hyper and diverse genus belongs to Solanaceae. It is represented in Kerala by about 33 species including those domesticated for their leaves, fruit vegetables or used as traditional medicine. *Solanum aculeatissimum* Jacq. commonly known as African night shade is used by the local people as fruit vegetable. Fresh healthy fruits of *S. aculeatissimum* were collected from the Western Ghats of Munnar hills, Kerala. Nutraceuticals properties of fruits were investigated and the results obtained were compared with other proven edible fruit plants. The major vitamins present in the fruits are thiamine (3.5 µg/g), riboflavin (1.8 µg/g), niacin (36.2 µg/g). Ascorbic acid level was (1.7 mg/g), pro-vitamin (166.2 µg/g). The essential minerals in the fruits were Fe (3.8), Ca (73) and Zn (14) in mg/100g (DW). Total protein and sugar levels were 4.11 ± 0.98 and 3.6 ± 1.3 mg/g tissue respectively. Further, the essential amino acid such as phenyl alanine and isoleucine were 33.63 and 40.22 µg/ g tissue. Anti nutritional factors in the fruits are phytic acid, and alkaloids. Trypsin and chymotrypsin protease inhibitors are present in substantial level, Total phenol was remarkably high i.e., 27.4 mg/g tissue, while flavonoid and tannin levels were 1.66 mg/g and 4.54 µg/ g tissue. The presence of these phytochemicals suggests that *S. aculeatissimum* may be potential source of many biological properties such as analgesic, anti-inflammatory, anti-hypertensive and anti-microbial. Despite the nutraceuticals, this species is still treated as invasive weed. Moreover, eco-friendly utilization of this species is already attempted in certain countries. The pharmaceutical and biotechnological companies should utilize this exotic fast growing *Solanum* with great commitments and need to address this bioresource.

Keywords: Nutraceuticals, Phenolic acids, Minerals, *S. aculeatissimum,* Protease Inhibitor.

INTRODUCTION

Plants possess tremendous sources of natural molecules which are used as pharmaceuticals, agrochemicals, flavour and fragrance agents, food additives and pesticides. Unraveling for novel phytochemicals should thus be a priority in current and future goals toward sustainable conservation and rational utilization of biological diversity. Edible crops such as vegetables and fruits are nowadays used for developmental nutritional research because of their potential to compete with conventional medicinal plants other than as nutritional ingredients or supplements. The metabolome of plants is a potent tool for small-molecule analysis aided by software innovations which enable identification and quantification of numerous phytomolecules. The metabolome of fruits thus makes them as resource of health bioactives for designer functional food of future.[1] Nutraceuticals can be defined as, "a food (or part of a food) that provides medical or health benefits, including the prevention and/or treatment of a disease".[2] Such foods also commonly are referred to as medical foods, nutritional supplements and functional foods thus signifying they and/or their components may provide a health benefit beyond basic nutrition. Nutraceuticals comprises of nutrients isolated from plants, dietary supplements, genetically modified foods and processed plant products. [3]

Solanum contributes the largest and most complex genus that composed of more than 2,000 species, many of which are also economically important. *Solanum* species represent nearly 1% of the world's angiosperm flora, which might be attributed to its great antiquity and an extraordinary rate of speciation. This huge diversity in one genus is quite exceptional in angiosperms, making *Solanum* interesting from an evolutionary standpoint as well as for its great economic importance. Examples of food plants in the Solanaceae are potato, eggplant and naranjilla (*S. psuedocapsicum*), jasmine nightshade (*S. jasminoides* Paxt.) and others.[4] Many species of *Solanum* are used as medicine like to cure digestive and intestinal problems, including stomach-ache, diarrhoea, piles, dysentery and for various skin problems such as sores, boils, cuts, wound and bruises. Many species are also employed to treat fever and malaria, headache and rheumatism. Some species are stimulants whereas others have sedative properties. Furthermore, many species are frequently used for various diseases of the respiratory tract, such as cough, sore throat, bronchitis, asthma and urinary problems. Similarly, *Solanum* shows insecticidal and fungicidal properties.[5] *Solanum aculeatissimum* Jacq. Commonly known as the African nightshade or Dutch egg plant. A shrubby perennial,

armed with spines, fruit are spherical, striped or marbled green and creamy-white, turning dirty yellow when ripe. The plant is native to Southern Africa but now widespread throughout Asia. The plant has been extensively used ethnically to cure various diseases such as constipation, purgative, back pain, male impotence, snakebites, toothache, headache, skin infections, flatulence, cough and dysmenorrhoea. Most of the medicinal attributes of the plant was due to the presence of steroidal glycoalkloids.[6] Despite its medicinal potentiality the plant is unrecognised and underutilized. With this aim the nutraceutical parameters of the "African Nightshade" i.e., *S. aculeatissimum*, is evaluated so that may be used as supplementary diet as well as medicine.

MATERIALS AND METHODS

Plant materials

Fresh fruits of *Solanum aculeatissimum* Jacq. was collected from Munnar hills, Kerala. The identity was confirmed with voucher specimen from Kerala Forest Research Institute, Peechi, Kerala (Fig. 1).

Fig. 1: Plant material - Solanum aculeatissimum Jacq.

Estimation of sugars

Total sugar content of fruits was estimated using DNS reagent and the absorbance of the reaction mixture was measured at 540 nm with spectrophotometer.

Estimation of Proteins

The soluble protein was estimated following the protocol of Lowry et al. [7] The absorbance was read at 670 nm after 30 min using appropriate blank and the amount of protein was calculated.

Total Flavonoids

Aluminium chloride colorimetric technique was used for flavonoid estimation. Aqueous and extract was mixed with 1.5 ml of methanol, 0.1 ml of 10% aluminium chloride, 0.1 ml of 1M potassium acetate and 2.8 ml of distilled water and was left at room temperature for 30 min after which the absorbance of the reaction mixture was measured at 415 nm with spectrophotometer.[8]

Alkaloid isolation

10 g coarsely powdered fruit was Soxhlet-extracted with 50 ml 2% methanolic acetic acid for 3 h, and the extract was diluted to 100 ml with 2% methanolic acetic acid. [9] 5 ml of the extract/solution was taken and the pH was maintained at 2–2.5 with dilute HCl. 2 ml amount of DR (Prepared by mixing (1) solution of 0.8 g bismuth nitrate pentahydrate in 40 ml distilled water and 10 ml glacial acetic acid, and (2) solution of 8.0 g potassium iodide in 20 ml distilled water) was added to it, and the precipitate formed was centrifuged. The centrifugate was checked for complete precipitation by adding DR. After centrifugation, the centrifugate was decanted completely and meticulously. The precipitate was further washed with alcohol. The filtrate was discarded and the residue was then treated with 2 ml disodium sulfide solution. The brownish black precipitate formed was then centrifuged. Completion of precipitation was checked by adding 2 drops of disodium sulfide. The residue was dissolved in 2 ml concentrated nitric acid, with warming if necessary. This solution was diluted to 10 ml in a standard flask with distilled water; 1 ml was then pipetted out, and 5 ml thiourea solution was added to it. The absorbance was measured at 435 nm against the blank containing nitric acid and thiourea. The amount of bismuth present in the solution was calculated by multiplying the absorbance values with the factor, taking suitable dilution factor into consideration. The factor is obtained from the standard curve, which is a constant for different concentrations.

Factor = concentration/absorbance

Quantification of total free amino acids

Total free amino acids were determined using the method of Moore and Stein.[10] 1 g fruit tissue was refluxed in 80% methanol for 10 min and homogenized. The homogenate was centrifuged at 7500 rpm for 10 min. The supernatant was collected and made to known volume using 80% methanol. 0.1 ml of the supernatant was mixed with 5 ml ninhydrin reagent. Shaken well and boiled for 10 min. The absorbance was read at 570 nm.

Estimation of Vitamins

Vitamin content of fruits was estimated by various standard spectrophotometric procedures.

Ascorbic acid

100 g tissue was ground using 10 ml distilled water, each time decanting off the liquid extract into a 100 ml volumetric flask. Extracted solution was made up to 100 ml with distilled water. Pipetted a 20 ml aliquot of the sample solution into a 250 ml conical flask and added about 150 ml of distilled water and 1 ml of starch indicator solution. Titrated the sample with 0.005 mol L^{-1} iodine solution. The endpoint of the titration was identified as the first permanent trace of a dark blue-black colour due to the starch-iodine complex. Titration was repeated to obtain concordant values.

α – Tocopherol

The tocopherol content of the samples was estimated by the method of IUPAC. [11] 1 g fruit tissue was refluxed in ascorbic acid and ethanol mixture for 3 to 5 min. 1 ml of potassium hydroxide and water mixture was added and boiled for 3 min. The mixture was cooled and 25 ml distilled water was added and transferred to a separating funnel. 50 ml of diethyl ether was added and shaken for 1 min. The lower aqueous layer was collected and washed three times with ether. The tocopherol content was dissolved in ether and the ether layer was washed with distilled water until it becomes alkali free. Ether layer was removed and the residue was redissolved in 5 ml of Benzene ethanol mixture and the extract was evaporated. The dry extract was then dissolved in 1 ml of hexane and was again evaporated. The residue was finally dissolved in 4 ml ethanol. The absorbance was measured at 292 nm and the tocopherol content was determined using the standard curve of α-tocopherol.

β –carotene

1 g of sample was taken in a stoppered conical flask and 20 ml of 60% potassium hydroxide was added and kept in the dark for 3 h for saponification. The mixture was then transferred to a separating funnel, washed with petroleum ether and the ether layer was collected. Washing was done for 6 times. The collected ether layers were combined and washed with distilled water until the layer became alkali free. The ether layer containing carotene was collected and made up to a known quantity and the absorbance was recorded at 429 nm with petroleum ether as blank.

Estimation of Anti nutritional factors

Protease inhibitor activity assay

Protease inhibitor activity was determined by estimating the residual hydrolytic activity of trypsin and chymotrypsin towards the substrates BAPNA (N-benzoyl-L-arginine-p-nitroanilide) and BTPNA (N-benzoyl-L-tyrosyl-p-nitroanilide), respectively, at pH 8.0 after pre-incubation with inhibitor.[12] One trypsin or chymotrypsin unit is defined as 1 µmol of substrate hydrolyzed per min of reaction. One inhibitor unit was defined as the quantity of inhibitor needed to inhibit 50% of the corresponding enzyme activity.

Estimation of Tannins and Phytic acid

Quantitative analysis of tannins was carried out by spectrophotometrically using Folins-Dennis reagent by the method of Singleton and Roos. [13]Extraction was done with methanol /water. Tannic acid was used to prepare the standard graph. Phytic acid content was determined by the method of Ravindran and Ravindran.[14]

Estimation of Total phenols

Total phenol content of fruits of *S. aculeatissimum* was estimated by the method of Mayr et al.[15] The reaction between phenol and an oxidizing agent phosphomolybdate in Folin – Ciocalteau reagent resulted in the formation of a blue complex. Standard graph of phenols was constructed with pyrocatechol by taking absorbance against concentration. The total phenol per gram tissue was calculated from the standard graph.

Estimation of metals

Open-vessel digestion procedure was used for preparation of plant digest for mineral composition analysis, using a Perkin Elmer 3110. The concentrations of different elements in these samples were determined by the corresponding standard calibration curves obtained. The essential mineral content in the fruits was estimated using standard atomic spectroscopy.

IR spectroscopy

The methanolic extract from fruits was used for IR fingerprinting. The samples were immediately dried in an oven for 2 days at 60°C. Tablets for FTIR spectroscopy were prepared in an agate mortars, by mixing leaves powder (2 mg) with KBr (1:100 p/p). The absorbance spectra were measured between 300 and 4500 cm^{-1}.[16] A FTIR spectrometer (FTIR Shimadzu Prestige 21) was used to collect spectra.

Statistical analysis

The mean of three replicates obtained from 3-5 independent experiments were calculated. All experimental data were analyzed by one-way analysis of variance (ANOVA). After confirming the significance of F values, the significance of the differences between the mean values was tested using ANOVA. Significant differences were considered at $p < 0.01$ probability levels.

RESULTS AND DISCUSSION

Soluble sugar

The amount of soluble sugar (3.6 ± 0.07 mg/g) in the present study was significantly lower. 5.45 mg/g and 17 mg/g sugar content was reported in *Solanum nigrum* and *S. tuberosum* respectively.[17]

Total protein

The protein level was comparable with other *Solanum sps.* i.e., 4.11 ± 0.976 mg/g. 5.2 ± 0.14 mg/g crude protein level was observed in *Solanum nigrum* and in *S. tuberosum* 20 mg/g protein was reported.[17]

Total flavonoid and alkaloids: The flavanoid content in the methanolic extraction was 1.66 ± 0.33 mg/g. Alkaloids (3.7 ± 0.21 mg/g) also showed comparatively low profile. Flavonoids are large class of polyphenols attributed to nutraceutical property in several plants. They are potent antioxidants and metal chelators.[18] Flavonoids possess multiple biological potentials like free radical scavengers, cellular signaling, antiinflammatory, antiallergic, antimicrobial, antiulcer, antiviral and antimetastatic and antihepatotoxic.[19]

Amino acid content: The essential amino acids like phenylalanine and isoleucine showed significant levels ie., shown in the table 1.

Table 1. Amino acid profile of fruit extract of *S. aculeatissimum*

AMINO ACIDS	(µg/g)
Tyrosine	485 ± 12.6
Phenylalanine	33.63 ± 0.56
Serine	77.62 ± 11
Glycine	34.79 ±4.8
Aspartic acid	233.3 ±10
Proline	330.35 ± 21
Cysteine	296 ± 11
Isoleucine	40.21± 0.07

Vitamin Analysis

Major vitamins noticed are thiamine (3.5 ± 0.45 µg/g), riboflavin (1.8 ± 0.66 µg/g), niacin (36.2 ± 0.25 µg/g). Ascorbate level was 1.7 ± 0.88 mg/g, pro-vitamin A 166.2 ± 0.064 µg/g and α- tocopherol 70 ± 0.044 µg/g. The values are at par with *S. torvum* where the vitamin content was, thiamine (1 µg/g), riboflavin (1.8 µg/g), niacin (36.2 µg/g), ascorbate (1.7 mg/g), pro-vitamin (166.2 µg/g) and α- tocopherol (70 µg/g).

Mineral content

The essential mineral content in the fruits are iron (3.8 ± 0.57), calcium (73 ± 0.43) and zinc (14 ± 0.31) in mg/100 g. Metal as micronutrient is important for the normal functioning of vital organs and is present in many enzymes which activate them, thereby influence the biochemical processes that are required in our diet. [17]

Anti nutritional factors

The activity of PIs isolated from fruits of *S. aculeatissimum* was evaluated. Protease inhibitor (Pl) unit obtained from fruits of *S. aculeatissimum* for trypsin (4865 ± 3.74 TIU) and chymotrypsin (4698 ± 6.05 CTIU) were comparatively higher.

Total phenol content showed remarkable level i.e., 27.4 ± 1.88 mg/g suggests the active phase of secondary metabolism and in turn the use of the plants as drugs. The high phenol content was also compared with other species such as *S. torvum*, *S.xanthocarpum* and *S.trilobatum* (15, 12.4 and 13.1 mg/g respectively).[20] The most active dietary antioxidants belong to the group polyphenolics. These polyphenolic are proven antioxidants and suggest that they are reported to quench oxygen-derived free radicals as well as the substrate-derived free radicals by donating a hydrogen atom or an electron to the free radical and the antioxidant activity of phenolics in several systems has indicated that they were as more active compared to synthetic antioxidants. Tannins (0.0045 ± 0.001 mg/g) and Phytic acid levels were comparatively less (123 ± 1.78 µg/g). Tannins are potent therapeutic agents, which can be used in neurological disorders.[21]

IR fingerprinting

The IR spectrum of fruit samples are shown in the Fig.2 with the most characteristic absorption bands and their tentative assignments are given in Table 2. The FT-IR spectrum exhibits the characteristic finger print band features. Total number of infra red peaks was 28. Prominent bands at 532.35 to 862.18^{-1} showed the alkyl halide groups. The IR spectra in the

sharp absorption peak at 1591. 27 cm^{-1} are assigned to aromatic groups. The presence of narrow and sharp peak at ~2115.91 cm^{-1} to ~2941.44 cm^{-1} was assigned alkanes. There are also specific peaks revealing the presence of phenols and alcohol (3307.92 – 3537.45cm^{-1}). The absorption bands and the major compounds are comparable with other *Solanum sps.*[22] In *Morinda citrifolia* the results of FTIR analysis confirmed the presence of polymeric hydroxyl groups, aromatic compounds, phenols and polyphenols and are attributed to their antioxidant effect.[23] The IR fingerprints in the range 3059 – 3122 represents the alkaloids the major drug compounds in *Solanum* species. The stronger the relative intensity of the band, the higher will be the chemical constituents.

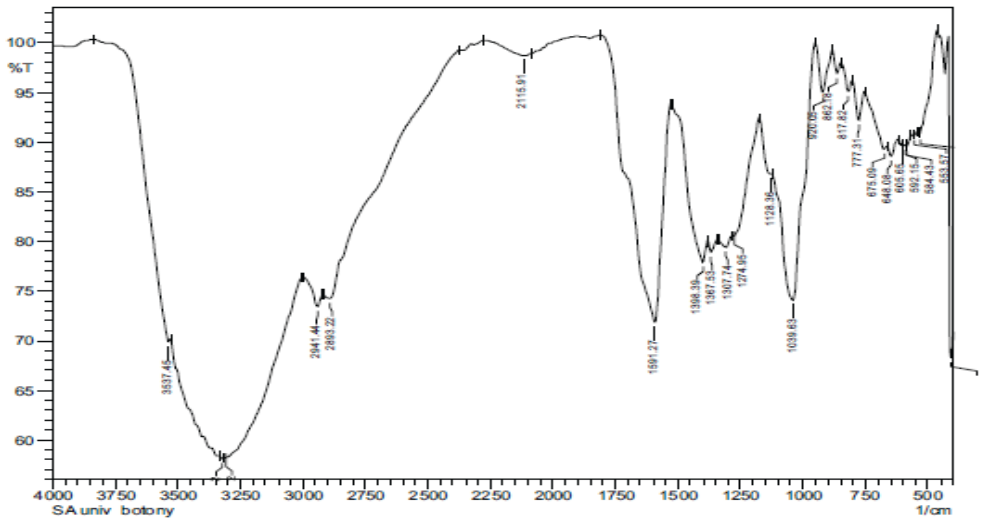

Fig. 2: FTIR spectra of methanol extract.

Table 2: FTIR spectra of methanol extract.

Peak range	Functional group	Peak range	Functional group
532.35	ALKYL HALIDES	1128.36	ALIPHATIC AMINES
553.57	ALKYL HALIDES	1274.95	AROMATIC AMINES
584.43	ALKYL HALIDES	1307.74	NITRO COMPOUNDS
592.15	ALKYL HALIDES	1367.53	ALKANES
605.65	ALKYL HALIDES	1398.39	NITRO GROUPS
648.08	ALKYL HALIDES	1591.27	AROMATICS
675.09	ALKYL HALIDES	2115.91	ALKYNES
777.31	ALKANES	2893.22	ALKANES
817.82	ALKYL HALIDES	2941.44	ALKANES
862.18	ALKYL HALIDES	3307.92	PHENOLS & ALCOHOLS
920.05	CARBOXYLIC ACIDS	3321.42	PHENOLS & ALCOHOLS
1039.63	ALIPHATIC AMINES	3537.45	PHENOLS & ALCOHOLS

CONCLUSION

The exploration of nutraceuticals (product isolated or purified from food) will accomplish desirable therapeutic products and this will meet the needs of the society. The potentiality and usability of *S. aculeatissimum* could be advanced if this shrub is used for the processing of nutraceuticals. The present study reveals the remarkable molecules in the fruits of *Solanum aculeatissimum* and its probable therapeutic potential and as nutrients. Therefore, it can be recommended for their consumption after further *in vivo* detailed studies.

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