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Pharmacognostical studies on *Solanum surrattense* Burm f. Root.

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Abstract

Objective: To investigate macroscopic, microscopic, qualitative phytochemical and pharmacognostic parameters of *Kantakari* (*Solanum surrattense* Burm f.) root, a plant species which is well mentioned in Ayurvedic classics and an important constituent of Ayurvedic combination forms i.e. *laghupanchmula* and *dashmula*. The plant is also used in preparation of variety of indigenous medicine. **Methods:** Macroscopic, microscopic, qualitative phytochemical analysis, physiochemical analysis, extractive values in ethanol and water of the root were done. **Results:** Macroscopic and microscopic study showed distinct morphological characteristics in the root. Physiochemical analysis of root powder revealed, moisture content 2.1%, total ash 10.4%, acid-insoluble ash 2.7%, sulphated ash 11.33%, alcohol soluble extractive 7.5%, water soluble extractive 13.6%. Alkaloids, carbohydrates, proteins, resins, saponins, steroid, tannin, starch, glycosides, flavonoids and triterpenoids were present in root extracts. **Conclusions:** pharmacognostic study of root is helpful in sample identification and to ensure quality and purity standards of *Kantakari* (*Solanum surrattense* Burm f.). The qualitative phytochemical screening is helpful in further pharmacological approaches.

Keywords: Kantakari, Pharmacognosy, phytochemical.

1. Introduction

Solanum surattense belongs to the family of Solanaceae. It is a commonly growing perennial herbaceous weed. In Hindi it is known as Katai, Katali, Ringani, Bhatakataiya, Chhotikateri and in English as Febrifuge plant, Yellow berried nightshade. *Solanum surattense* is distributed throughout India, Sri Lanka, South East Asia, Malaysia and tropical Australia. It has been used traditionally for curing various ailments such as fever, cough, asthma and diabetes in south Indian traditional medicines. The antidiabetic potential of the fruit was studied in diabetic rats^[1, 2]. The ethanol and methanol extracts of *S. surattense* showed strong antibacterial activity against *Pseudomonas aeruginosa*^[3]. Wound healing activity^[4], physicochemical activity^[5] and antioxidant potential^[6] of the plant is also evaluated.

2. Materials and Methods

2.1. Plant material

The root of *Kantakari* (*Solanum surrattense* Burm f.) was collected from Rajiv Gandhi South Campus, Banaras Hindu University, Mirzapur in months of November-December, 2011 and identified with the standard sample (no. Dg1001-DG1006) preserved in the department of Dravyaguna, Institute of Medical Sciences, Banaras Hindu University, Varanasi.

3. Macroscopic and microscopic analysis

The root was studied macroscopically for important identification points, i.e. odour, taste and texture and for microscopic studies; a transverse section was prepared and stained. Microscopy of power was investigated according to method of Kokate (2010)^[7].

Macroscopic Evaluation

3.1. Size

A graduated ruler in millimeters was used for the measurement of the length, width and thickness of crude materials.

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Fig 1: Whole Plant of *Solanum surattense* Burm. f.



Fig 2: Macroscopic characteristic of *Solanum surattense* Burm.f. Root.

3.2. Colors

The untreated sample was examined under the diffused daylight. The color of the sample was compared with that of a reference sample.

3.3. Surface characteristics, texture and fracture characteristics

The untreated sample was examined for organoleptic characters. The material was touched to determine if it is soft or hard, it was made bend as ruptured to obtain information on brittleness and the appearance of the fracture plane - whether it was fibrous, smooth, rough, granular etc.

3.4. Odour

The sample was placed on the palm of the hand and slowly and repeatedly air was inhaled over the material. Further, the sample was rubbed between the thumb and index finger or between the palms of the hands using gentle pressure. The strength of the odour (none, weak, distinct, strong) and then the odour sensation (aromatic, fruity, musty, rancid etc.) was determined. A direct comparison of the odour with a defined substance was done.

4. Powder microscopy

Some of important characters for root identification are cork cells, cortex, xylem, phloem, vessels, and vascular bundles, starch grain, calcium oxalate crystals; etc which is observed in powder microscopy. Powder microscopy was performed as per WHO guidelines where 1 or 2 drops of water, glycerol/ethanol or chloral hydrate was placed on a glass slide. The tip of a needle was moisten with water and was dipped into the powder. A small quantity of the material that adheres to the needle tip was transferred into the drop of fluid on the slide. It was stirred thoroughly, but carefully, and a cover-glass was applied. The cover-glass was pressed gently with the handle of the needle, and excess fluid was removed from the margin of the cover-glass with a strip of filter-paper^[8].

5. Microscopy of root by Microtoming Method

Microtoming is a method to obtain fine and thin sections of plant tissue ranging from 5-12 μ m. It is a multistep process as described below^[8].

5.1. Collection of material, killing and fixing

Reagents used for killing and fixing includes, absolute ethyl alcohol, 95% ethyl alcohol, chloroform, glacial acetic acid and formalin. The amount of killing and fixing fluid was 10 times the volume of material.

5.2. Washing

All aqueous fluids, particularly those containing chromic acid were washed out with water. Alcoholic solutions were washed out with plain alcohol. Reagents containing picric acid whether in aqueous and alcoholic solution were washed with alcohol.

5.3. Dehydration

After washing, the next step is to remove the water from the tissue. Tertiary butyl alcohol was used for dehydration. Different series of water, ethyl alcohol and tertiary butyl alcohol was prepared. After dehydration, the tissue was ready for infiltration and transferred to paraffin. It was placed in paraffin for one hour.

5.4. Embedding

The tissue was poured in the paraffin oil mixture into suitable receptacles/mold and quickly cooled. After the paraffin block was cooled it was removed from the mold and trimmed with a scalpel and left overnight for cooling.

5.5. Microtoming

For obtaining thin sections microtome was used through which fine sections were obtained ranging from 3-12 μ m.

5.6. Mounting

The ribbon was cut into sections of convenient length. Adhesive material was rubbed on the slide for affixing sections to the slide. The ribbon containing section was placed on the slide and flooded with distilled water and the ribbon was allowed to get straightened out. The slide is then put on hot plate with temperature 43 °C and is left for overnight

5.7. Staining

After the section was fixed on the slide it was then preceded for staining. Before staining the paraffin was removed by using xylene. The slide was placed in jar containing xylene for about 5 minutes, after this the slide was passed through ethyl

alcohol solutions of different concentration in increasing order which is finally washed with absolute alcohol followed by xylene. The slide was then allowed to dry in air. After this process the sections were subjected to staining using staining reagent. After staining sections were mounted with DPX so as to prepare permanent slide.

6. Physiochemical analysis

Physiochemical studies such as moisture content, total ash, foreign matter, acid insoluble ash, sulphated ash were determined according to WHO guidelines on quality control methods for medicinal plants [9].

6.1. Determination of Foreign Matter

100 g of the powdered sample of the crude drug was weighed accurately. It was then spread in a thin layer on a white tile uniformly without overlapping and the inspection was done with naked eyes or with the help of a 6X lens. The foreign matter was then separated manually as completely as possible. The portions of this sorted foreign matter were weighed and the content of each group in grams per 100 g of air-dried sample was calculated.

6.2. Determination of Loss on Drying

A glass-Stoppard, shallow weighing bottle was weighed, accurately 2 g of the specified sample was weighed and then transferred to the bottle and the bottle containing the content was again accurately weighed. The sample was then distributed as evenly as practicable by gentle sidewise shaking to a depth not exceeding 10 mm. The loaded bottles were placed in the drying chamber (hot air oven). Eventually the sample was then dried to constant weight for the specified time and at the temperature (over 110 °C). After the drying was completed the bottle was promptly removed and allowed to cool at room temperature in desiccators before weighing. Finally the weight of the bottle along with the contents was weighed and the percentage loss in weight was calculated.

6.3 Determination of Ash Value

Total Ash

Accurately 2 g of the ground, air-dried material was weighed and placed in a previously ignited and tarred silica crucible. The powder sample was then spread evenly and then was incinerated to a constant weight by gradually increasing the temperature to 500-600 °C until it was white, indicating the absence of carbon. The crucible was then cooled in a desiccator and finally weighed. The content of total ash in terms of percentage w/w of air dried material was calculated.

Acid Insoluble Ash

The crucible containing the total ash obtained after incineration was further boiled gently for 5 minutes with 25 ml of hydrochloric acid (~70 g/litre) and was then covered with a watch glass. The watch glass was then rinsed with 5 ml of hot water and this liquid was then added to the crucible. The insoluble matter was then collected on an ashless filter paper and washed with hot water until the filtrate was neutral. The filter paper containing the insoluble matter was then transferred to the crucible, dried on a hot-plate and then was ignited to constant weight. The residue was allowed to cool in a suitable desiccator for 30 minutes, and then weighed without delay. The content of acid-insoluble ash was calculated in mg per gram of air-dried material.

Water Soluble Ash

The crucible containing total ash, 25 ml of water was added and boiled for 5 minutes. The insoluble matter was then collected on an ash less filter paper. It was then washed with hot water and finally ignited in a crucible for 15 minutes at a temperature not exceeding 450 °C. The percentage of water soluble ash in mg per gram of air-dried material was reported.

6.4 Extractive Value

4.0 g of coarsely powdered air-dried material was accurately weighed and placed in a glass-stoppered conical flask. Powder was then macerated with 100 ml of the solvent (Water/ethanol) concerned for 6 hours, shaking frequently, and then was allowed to stand for 18 hours. It was then filtered rapidly taking care not to lose any solvent; 25 ml of this filtrate was transferred to a tarred flat-bottomed dish and was evaporated to dryness on a water-bath. It was followed by drying at 105 °C for 6 hours, cooled in a desiccator for 30 minutes and was weighed without delay. The content of extractable matter in mg per g of air-dried material was then calculated.

7. Preliminary Phytochemical Screening of extracts

50% ethanolic extracts were obtained by cold maceration and they were subjected to preliminary phytochemical screening for the presence of various phytoconstituents using various qualitative reagents [10].

7.1 Alkaloids

Preparation of test solution: The test solution was prepared by dissolving extracts in the dilute hydrochloric acid solution.

- 1. Mayer's test:** The acidic test solution with Mayer's reagent (Pot. Mercuric iodide) gave cream coloured precipitate.
- 2. Dragendorff's test:** The acidic solution with Dragendorff's reagent (Potassium bismuth iodide) showed reddish brown precipitate.
- 3. Wagner's test:** The acidic test solution treated with Wagner's reagent (Iodine in Potassium iodide) gave brown precipitate.

7.2 Amino Acids

- 1. Test for Cysteine:** To 5 ml test solution few drops of 40% NaOH and 10% lead acetate solution were added and boiled, black precipitate of lead sulphate was formed.

7.3 Carbohydrates

Preparation of test solution: The test solution was prepared by dissolving the test extract with water. Then it was hydrolyzed with 1 volume of 2N HCl and subjected to following chemical tests.

- 1. Fehling's test:** The test solution when heated with equal volume of Fehling's A and B solutions, gave orange red precipitate, indicating the presence of reducing sugars.
- 2. Tests for pentoses:** The test solution with equal volume of HCl containing little phloroglucinol was heated. Formation of red colour indicated presence of pentose.
- 3. Test for Hexoses:** 3 ml test solution with 2 ml cobalt

chloride solution was mixed, boiled, cooled. Few drops of NaOH solution was then added. Solution appeared greenish blue (glucose) or purplish (fructose) or upper layer greenish blue and lower layer purplish (mixture of glucose and fructose).

7.4 Flavonoid

1. Preparation of test solution:

- To a small amount of extract, equal volume of 2M HCl was added and heated in a test tube for 30 to 40 min. at 100 °C.
- The cooled extract was filtered, and extracted with ethyl acetate.
- The ethyl acetate extract was concentrated to dryness, and used to test for flavonoids.

- **Shinoda test:** Test solution with few fragments of magnesium ribbon and conc. HCl showed pink to magenta red colour.
- **Alkaline reagent test:** Test solution when treated with sodium hydroxide solution showed increase in the intensity of yellow colour, which becomes colourless on addition of few drops of dilute acid.

7.5 Anthraquinones Glycosides

- **Preparation of test solution:** The test solution was prepared by dissolving extract in the alcohol or hydro-alcoholic solution.
- **Borntrager's test:** Powdered drug with 5 ml of 10% sulphuric acid was boiled for 5 min. Filtered while hot, cooled; the filtrate was shaken gently with equal volume of benzene. Benzene layer was separated and then treated with half of its volume solution of ammonia (10%) and was allowed to separate. The ammoniacal layer acquired rose pink colour due to the presence of anthraquinones.

7.6 Proteins

- **Biuret test:** Test solutions were treated with 40% sodium hydroxide and dilute copper sulphate solution which gave blue colour.
- **Xanthoproteic test:** Test solution was treated with conc. HNO₃ and boiled which gave yellow precipitate.

7.7 Saponin

Foam test: Test solution when shaken with water showed formation of foam, which was stable for at least 15 min confirms saponin.

7.8 Steroids

Preparation of test extract solution: The extracts were refluxed separately with alcoholic solution of potassium hydroxide till complete saponification. The saponified extract was diluted with water and unsaponifiable matter was extracted with diethyl ether. The ethereal extract was evaporated and the residue (unsaponifiable matter) was subjected to the following test by dissolving the residue in the Chloroform.

Salkowski test: To the test extract solution add few drops of conc. H₂SO₄, shaken and allowed to stand, lower layer turned

red indicating the presence of sterols.

7.9 Tannins and phenol compounds

To 2-3 ml of alcoholic or aqueous extract, added few drops of following reagents:

- **5% FeCl₃ solution:** Deep blue-black colour.
- **Lead acetate solution:** White precipitate.
- **Acetic acid solution:** Red colour solution.
- **Dilute iodine solution:** Transient red colour.

8. Observations and Results

8.1. Macroscopic

Root is long, cylindrical and tapering, bearing a number of fine longitudinal and few transverse wrinkles with occasional scars or a few lenticels and small rootlets, transversely smoothed surface shows a thin bark and wide compact cylinder of wood, fracture, short, taste, bitter.

8.2. Microscopic

Transverse section of mature root shows cork composing of thin-walled, rectangular and tangentially elongated cells, cork cambium single layered followed by layers of thin-walled, tangentially elongated to oval or circular parenchymatous cells, stone cells either single or in groups, secondary phloem composed of sieve elements and phloem parenchyma traversed by medullary rays, xylem composed of vessels, tracheids, fibre tracheids, parenchyma and transverse by medullary rays, all elements being lignified, vessels and tracheids with bordered pits, fibres with a few simple pits, xylem parenchyma rectangular or lightly elongated with simple pits and rarely with reticulate thickening, crystals of calcium oxalate as sandy masses and simple starch grains present in secondary cortex, phloem and medullary rays (Figure 3).

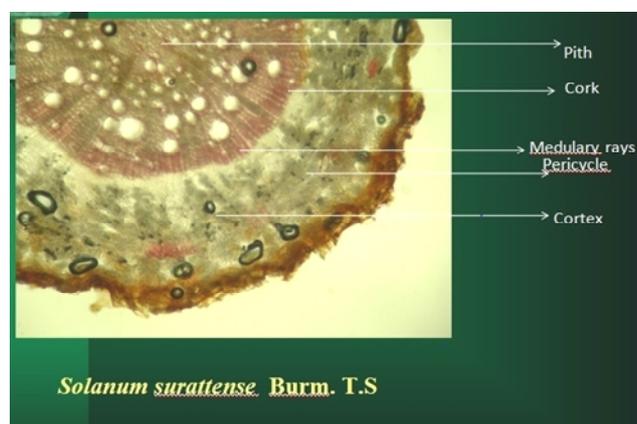


Fig 3: Microscopic characteristics of *Solanum surattense* Burm. f. root. T.S. of root showing pith, cork, medullary rays and pericycle.

Root powder is cream in colour, having no specific odour and taste, it is fibrous in texture. Powder revealed abundant simple and compound starch grains in entire section which were rounded to oval. With phloroglucinal and concentrated hydrochloric acid it gave the appearance of fibrovascular bundles (wood elements). Calcium oxalate crystals and stone cells were also seen (Figure 4).

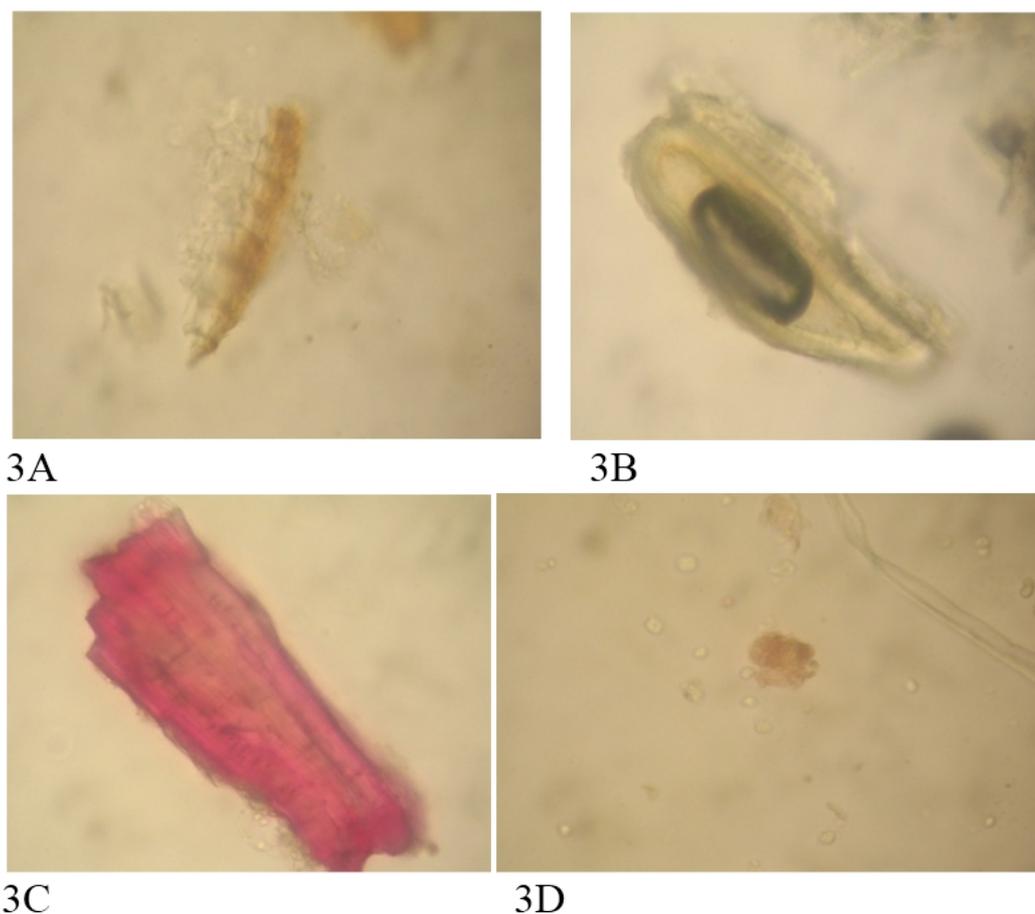


Fig 4: Powder characteristics of *Solanum surratense* Burm. f. root.

3A: Cork cells; **3B:** Stone cell; **3C:** Vascular element; **3D:** simple and compound starch grains and calcium oxalate crystal

8.3. Physicochemical Parameters

Physicochemical studies such as moisture content, total ash, foreign matter, acid insoluble ash and sulphated ash were presented in table 1.

Table 1: Physicochemical characteristics of *Kantakari* (*Solanum surratense* Burm f.) Root.

Parameters	Values
Foreign matter	2%
Moisture content	2.1%
Total ash	10.4%
Acid- insoluble ash	2.7%
Sulphated ash	11.33%
Alcohol soluble extractive	7.5%
Water soluble extractive	13.6%

8.4. Phytochemical Screening

Table 2: Qualitative Phytochemical Screening of plants of *Kantakari* (*Solanum surratense* Burm f.) Root.

Chemical constituents	Chemical tests	Aqueous extract	Ethanollic extract
<i>Alkaloids</i>	<i>Dragendroff test</i> <i>Hager's test</i> <i>Wagner's test</i> <i>Mayer's test</i>	+	+
<i>Carbohydrate</i>	<i>Fehling's test</i> <i>Anthrone test</i>	+	+

	<i>Benedict's test</i> <i>Molisch's test</i>		
<i>Flavonoids</i>	<i>Shinoda's test</i>	+	+
<i>Triterpenoids</i>	<i>Liebermann Burchard test</i>	+	+
<i>Proteins</i>	<i>Biuret's test</i>	+	+
<i>Saponins</i>	<i>Foam test</i>	+	+
<i>Steroid</i>	<i>Liebermann Burchard test</i>	+	+
<i>Tannins</i>	<i>Ferric chloride test</i>	+	+
<i>Starch</i>	<i>Iodine test</i>	+	+
<i>Glycoside</i>	<i>Keller-Kiliani test</i>	+	+

+: Present; -: Absent

9. Conclusions

Root of Kantakari (*Solanum surratense* Burm f.) was subjected to systemic microscopic, physicochemical and preliminary phytochemical analysis. The data generated is helpful in determining the quality and the purity of the drug, especially in the crude form. The extractive values are being useful for the further extraction of phytoconstituents from the plant. The alcohol soluble extractive indicated the presence of polar constituents like phenols, flavonoids etc. The total ash is particularly important in the evaluation of purity of drugs i.e. the presence or absence of foreign matter such as metallic salts or silica. The preliminary phytochemical screening of the extract of root was found to contain alkaloids, carbohydrates, glycosides, proteins, flavonoids, triterpenoids, steroids, tannins and saponins.

10. Conflict of interest statement

We declare that we have no conflict of interest.

11. Acknowledgement

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