Macro-microscopic evaluation and HPTLC-densitometric analysis of solasodine from fruits of some medicinally important species in genus Solanum Linn.

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Fruits of Solanum Linn. (Family — Solanaceae) species, viz. S. anguivi Lam., S. nigrum Linn., S. melongena Linn., S. virginianum Linn. and S. torvum Sw. are used in Indian traditional system of medicine for diverse pharmacological activities. These fruits are foremost source of solasodine which is one of the major starting materials used for commercial synthesis of steroidal drugs. 

In the present study, comparative micro-morphology of fruits of above five species and estimation of active principle solasodine with HPTLC-densitometric method has been carried out. An artificial key based on macroscopic and microscopic characters were developed for identification and authentication of fruits. The trend of solasodine content was as: S. virginianum > S. nigrum > S. torvum > S. anguivi > S. melongena. This data is helpful in identification and authentication of plant samples and quantification of the active marker solasodine.

Keywords: Authentication, Fruits, HPTLC, Solanum sp., Solanaceae, Solasodine, Steroidal drugs.

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Introduction

Herbal medicines have become the most sensitive topic all over the world due to dissatisfaction with unwanted effects of synthetic medicines. It led to the increasing demand for herbal resources and awareness for maintaining quality and purity of the raw materials1. At other side search of valid alternative for authentic drug is going on to avoid over exploitation of herbal resources from their natural habitat which may increase chances of adulteration and substitution in herbal material. Correct botanical identification by using taxonomic, macroscopic, organoleptic, microscopic studies, powder analysis and thin layer chromatography profiling of chemical constituents of respective raw material is one of the crucial parameter to ensure authenticity, quality, safety and efficacy of herbal products2.

The genus Solanum Linn. (Family — Solanaceae) is a part of the traditional system of Indian medicine with long history of use for treatment of wide array of disorders. It is well known for the presences of C27 — steroidal alkaloids. Fruits of different species like S. anguivi Lam., S. nigrum Linn., S. melongena Linn., S. virginianum Linn. and S. torvum Sw. are commercially sold in raw drug market in crumpled or powder form and used as basic constituent for various formulations3,4. They are major resources of solasodine and its major precursors5-7. Solasodine has been reported for various ailments like asthma, liver diseases, fevers, inflammation and sex hormone imbalances8,9.

Perusal of literature showed reports on micro-morphological characters of seeds of S. nigrum10 and S. melongena11. Several analytical methods including HPTLC12,13 for analysis of solasodine from fruits of S. virginianum7,14 S. nigrum5, S. torvum15, S. khasianum C. B. Clarke, S. gracile Otto ex Baxter, S. laciniatum Aiton and in formulation12 were reported. It has also been quantified in biological tissues16-18 but data is lacking for botanical standardization. However, in view of its diverse medicinal applications and growing demand from herbal drug market scientific validation and technological standardization is of prime importance.

In present investigation five commercially sold Solanum species were collected and comparative macroscopic, microscopic characters were studied
and estimation by HPTLC method for quantitative analysis of biologically active compound solasodine was carried out. An artificial key based on macroscopic and microscopic characters were developed for identification and authentication of fruits.

**Materials and Methods**

**Macroscopic and microscopic studies**

Fruits of *S. anguivi* (SA), *S. melongena* (SM) and *S. virginianum* (SV) were collected from crude drug market at Pune, Maharashtra, India. *S. nigrum* (SN) was collected from botanical garden at Agharkar Research Institute (ARI) and *S. torvum* (ST) was collected from Kolhapur - Belgaon highway, India (Plate 1). These fruits were authenticated and deposited at Repository, Botany Group, Agharkar Research Institute vide voucher specimen numbers *S. virginianum* F-148, *S. anguivi* F-149, *S. melongena* F-150, *S. torvum* F-151, *S. nigrum* F-152. Samples collected were shade dried and powdered, passed through 80-mesh sieve; stored in an airtight container at 25°C and used for further studies.

The macroscopic and microscopic studies were carried out as per WHO guidelines\(^{19}\). For powder microscopy, samples were washed with water to remove sugar and cleared by heating gently with saturated choral hydrate solution, cooled and mounted in 50% glycerin for microscopic observation. Microscopic documentation was carried out using Leitz Laborlux D research microscope\(^{20}\).

**Quantitative analysis of HPTLC**

*Chemicals:* Standard solasodine (99%) was gifted by In-Charge, Chemistry Group, Agharkar Research Institute, Pune, Maharashtra, India. Silica gel F\(_{254}\) HPTLC plates were purchased from Merck, Darmstadt, Germany. Other analytical grade solvents and reagents were purchased from S. D. Fine Chem Ltd., Mumbai, India.

*Preparation of standard solution:* A stock solution of solasodine (1mg/ml) was prepared in methanol.

*Chromatographic conditions*

HPTLC was performed on aluminium backed HPTLC plates 10 × 10 cm coated with 0.2 mm layers of silica gel 60 F\(_{254}\) (E. Merck, Germany).

The samples were applied on plate with band width 6 mm employing Linomat IV sample applicator (Camag, Switzerland) fitted with a microliter syringe. Linear ascending development of the plates up to distance of 80 mm was performed with mobile phase chloroform:methanol (7.5:1.2, v/v) in twin-trough glass chamber previously saturated with mobile phase vapour for 10 min at 25°C. The dried plates were derivatized by anisaldehyde sulphuric acid reagent and scanned at wavelength of 546 nm using a Camag TLC scanner 3 with CATS 4 software.

**Preparation of calibration curve**

Aliquot 0.02 ml of stock solution was transferred to volumetric flask and diluted to 10 ml with methanol to furnish working standard solution. Working standard solutions 2, 4, 6, 8, 10 and 12 µl of different concentrations; 40, 80, 120, 160, 200 and 240 ng, respectively were prepared by diluting stock solution. Each solution (20 µl) was applied on the plate and the plate was developed under predetermined condition described above. This procedure was repeated thrice to plot a graph of peak area and concentration of solasodine. A linear regression coefficient (r) for the standard confirms the linearity of the method and amount of solasodine.

**Preparation of sample solution**

Dried powders of respected fruits (2 g) were extracted with 150 ml of methanol in a Soxhlet apparatus for 18 hours. Remove the methanol by distillation under reduced pressure to yield the SA (0.1 g), SM (0.08 g), SN (0.1 g), ST (0.2 g), SV (0.13 g) crude residues, respectively. Take 5 mg of respected residues and dissolve in 10 ml of ethanol. Use these solutions for HPTLC profiling.

**Quantification of solasodine in drug sample**

Suitably diluted sample solution (5 µl) was applied in triplicate on a HPTLC plate along with standard. The plate was developed under predetermined conditions and scanned at 546 nm for solasodine. The peak areas and absorption spectra were recorded. To check the identity of the bands, the UV absorption spectrum of each standard was overlaid with the corresponding band in the sample track. The purity of the bands in the sample extract was checked by overlaying the absorption spectra at start, middle and end positions of the bands. The amount of solasodine in the sample was calculated using the linear regression equation derived from the calibration curves.

**Results and Discussion**

**Macroscopic and microscopic studies**

**Macroscopy**

The fruits of selected Solanum species are berries, fleshy, globose, smooth, with persistent calyx, the calyx with or without thorns; seeds flat, discoid, yellow. The size and surface appearance of the fruit as well as seeds; and characters of calyx will be helpful to differentiate respective Solanum species. The comparative differentiating macroscopic characters are formulated in Table 1. By using above observations the diagnostic key is developed for identification of materials.

**Key based on macroscopic characters**

1. Persistent calyx with thorn
2. Seeds more than 3.5 mm
   3. Fruit more than 1.2 cm in diameter ………SM
   4. Fruits less than 1.2 cm in diameter …………SA
2. Seeds less than 3.5 mm……………………..SV
1. Persistent calyx without thorn.
   4. Mature fruits brown and more than 1.2 cm in diameter ……ST
   4. Mature fruits red to black and less than 1.2 cm in diameter……..SN

**Microscopy**

Powder analysis of fruit of all five mentioned species shows very similar characters. The epicarp cells differ in their sizes and presence or absence of lumen. The spermoderm cells of seeds are specifically arranged on the surface giving it mosaic appearance. It forms irregular cavities on seed surface. In transverse view, these cells are palisade like and elongated. The size and arrangement of these cells are different in respective Solanum species. The diagnostic characters of powder are formulated in Table 2 and Fig. 1. By using above observations the diagnostic key is developed on the basis of microscopic characters for identification of crude materials.

**Key based on microscopic characters**

1. Epicarp cells more than 45 µm in diameter in surface view
2. Palisade-like cells of testa less than 20 µm thick………………………… SN
2. Palisade-like cells of testa more than 20 µm thick………………………… SM
1. Epicarp cells less than 45 µm in diameter in surface view
3. Palisade-like cells of testa more than 100 µm in height
4. Cavity form by spermoderm cells more than 200 µm in length
4. Cavity form by spermoderm cells less than 200 µm in length
3. Palisade-like cells of testa less than 100 µm in height

**HPTLC studies**

Of various solvent systems tried, chloroform: methanol (7.5:1.2, v/v) gave optimum results with resolution of solasodine at Rf 0.33 in the presence of other components in the sample extracts. The calibration plot shown in Fig. 2 indicates that the concentrations range of solasodine 40-240 ng was linear function and correlation coefficient was 0.9924 (Y= 354.29X + 626.67).

**Table 1—Comparative macroscopic characters of fruits of selected Solanum species**

<table>
<thead>
<tr>
<th>Name of Species</th>
<th>Fresh fruit</th>
<th>Dry fruit</th>
<th>Seed</th>
<th>Seed colour and size</th>
</tr>
</thead>
<tbody>
<tr>
<td>Solanum virginianum</td>
<td>Berry, globular, smooth, green, occasionally with white strips, mature yellow, 1.5 - 2 cm in diam., persistent calyx with spines</td>
<td>Yellow, smooth, not shiny, rarely wrinkled</td>
<td>Discoid, flat, slightly reniform</td>
<td>Yellow to brownish yellow, 1.8 - 2.5 mm in diam.</td>
</tr>
<tr>
<td>Solanum anguivi</td>
<td>Berry, globular, green, occasionally with white strips, mature light yellow, about 1.2 cm in diam., persistent calyx without spines</td>
<td>Light yellow, shiny frequently wrinkled</td>
<td>Oval to round, discoid</td>
<td>Yellow to yellowish brown, about 4 - 5 mm in diam.</td>
</tr>
<tr>
<td>Solanum torvum</td>
<td>Berry, globular, smooth, green, mature yellow to brownish yellow, about 1.3 cm in diam., persistent calyx without spines, shiny</td>
<td>Yellowish brown to brown, shiny, frequently wrinkled</td>
<td>Oval to round, discoid, slightly reniform</td>
<td>Yellow to yellowish brown, 1.5 - 2 mm in diam.</td>
</tr>
<tr>
<td>Solanum melongena</td>
<td>Berry, globose to oval, smooth, persistent calyx with spines, about 4 - 10 cm long and 4 - 7 cm in diam. Green, occasionally with white strips, sometimes dark violet at maturity, shiny</td>
<td>Fruits brown to dark brown or violet, frequently wrinkled</td>
<td>Oval to round, discoid, slightly reniform</td>
<td>Yellow to brown, 2 - 3 mm in diam.</td>
</tr>
<tr>
<td>Solanum nigrum</td>
<td>Berry globose to round, smooth, shiny, persistent calyx without spines about 0.6 to 1 cm in diam., green, mature red to black</td>
<td>Black, frequently wrinkled</td>
<td>Oval to reiform</td>
<td>Yellow, 1 to 2 mm in diam.</td>
</tr>
</tbody>
</table>

**Table 2—Diagnostic characters of comparative powder microscopy of fruits of selected Solanum species**

<table>
<thead>
<tr>
<th>Name of Species</th>
<th>Powder characteristics</th>
<th>Epicarp cells (Surface view)</th>
<th>Spermoderm cells (Surface view)</th>
<th>Spermoderm cells (Transverse view)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Solanum virginianum</td>
<td>Yellow to cream coloured, slightly gritty</td>
<td>With lumen, 20-30 µm in diam</td>
<td>Irregular, more regular and larger at peripheral side of seed, cavity 150-250 long</td>
<td>Palisade like, 65-100 µm in height, 20-30 µm in width</td>
</tr>
<tr>
<td>Solanum anguivi</td>
<td>Light yellow coloured, gritty</td>
<td>With narrow lumen, occasionally filled with yellowish content, 15-25 µm in diam</td>
<td>Irregular, cavity 100 to 150 µm long</td>
<td>Palisade like, 120-180 µm in height, 40-60 µm in width</td>
</tr>
<tr>
<td>Solanum torvum</td>
<td>Dark yellow or brown, gritty</td>
<td>With lumen, 20-35 µm in diam</td>
<td>Irregular, cavity 200-280 µm long</td>
<td>Palisade like, 100-125 µm in height, 40 - 50 µm in width</td>
</tr>
<tr>
<td>Solanum melongena</td>
<td>Dark brown with white particles, gritty</td>
<td>With broad lumen, 50-60 µm in diam</td>
<td>Irregular, more regular and larger at peripheral side of seed, cavity 250-350 µm long</td>
<td>Palisade like, 70-90 µm in height, 20-30 µm in width</td>
</tr>
<tr>
<td>Solanum nigrum</td>
<td>Dark brown to black coloured</td>
<td>Without lumen, with longitudinal markings, 75-95 µm diam</td>
<td>Regular, polygonal with large lumen, cavity 100-150 µm long</td>
<td>Palisade like, narrow, 70-80 µm in height, 10-15 µm in width</td>
</tr>
</tbody>
</table>
Fig. 1—Diagnostic characters of comparative powder microscopy of fruits of selected *Solanum* species
Fig. 2—Calibration curve indicates concentration range of Solasodine 40 ng to 240 ng ($r^2 = 0.9924; Y= 354.29X + 626.67$)

Fig. 3—HPTLC chromatograms of fruit drug extracts scan at 540 nm. (a-e samples; 1-6 standard solasodine): a- SM, b- SA, c- ST, d- SN, e- SV; 1- 40 ng; 2- 80 ng; 3- 120 ng; 4-160 ng; 5- 200 ng; 6- 240 ng

The method was specific for solasodine because it resolved the compound at $R_f$ 0.33. The identified solasodine band from samples extract was confirmed by overlaying UV absorption spectrum of samples with standard at 546 nm (Fig. 3). The comparative chromatogram of above described species with standard solasodine is done (Fig. 4). The amount of solasodine contents present in fruit samples of various *Solanum* spp. was calculated from calibration curve presented in Table 3.

**Conclusion**

Macroscopic and microscopic markers are helpful for identification and authentication of fruits of selected medicinally important species of genus *Solanum*. The artificial key developed on the basis of macroscopic and microscopic characters. The HPTLC quantification of the phytochemical reference standard solasodine in the fruits of different *Solanum* species revealed that the amount varied in five species. Diagnostic macro-microscopic character and

**Table 3—Comparative Solasodine content of samples of fruits of selected *Solanum* species**

<table>
<thead>
<tr>
<th>Name of Species</th>
<th>Solasodine (mg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Solanum virginianum</em></td>
<td>1.85±0.02</td>
</tr>
<tr>
<td><em>Solanum nigrum</em></td>
<td>0.94±0.05</td>
</tr>
<tr>
<td><em>Solanum torvum</em></td>
<td>0.82±0.02</td>
</tr>
<tr>
<td><em>Solanum anguivi</em></td>
<td>0.09±0.04</td>
</tr>
<tr>
<td><em>Solanum melongena</em></td>
<td>0.065±0.07</td>
</tr>
</tbody>
</table>

HPTLC profile of all the species and the amount of solasodine may play a significant role in identification and quality evaluation of different species of *Solanum* used in compound formulations.

**Acknowledgments**

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**References**

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