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Research Paper

# Identification of bioactive compounds in Solanum incanum fruit by Thin layer Chromatography and HPTLC

Biochemistry

**KEYWORDS :** Solanumincanum , Thinlayer Chromatography (TLC), High Performance Thin layer Chromatography (HPTLC).

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## ABSTRACT

*Solanumincanum fruit was evaluated for its secondary metabolites using standard analytical methods like and Thin layer Chromatography (TLC) technique and High Performance Thin layer Chromatography (HPTLC). Preliminary phytochemical analysis of fruit extract showed the presence of Alkaloids, Flavonoids, Carbohydrates, Glycosides, Phenols, Steroids, Tannins, Resins and Proteins. In this present study, Thinlayer Chromatographic analysis showed the presence of alkaloids, Flavonoids, Steroids, Glycosides and triterpenoids. After conformation with the presence of Alkaloids and Flavonoids, Solasodine and Quercetin were analyzed and compared with their standards by HPTLC.*

**Introduction**

Solanum incanum commonly known as bitter garden egg belongs to the family Solanaceae. It is a delicate perennial often cultivated as an annual crop. It is a shrub, growing 1 – 3m high. The leaves are simple, ovate, elliptic, 2.5 – 12 cm long and 2.5 – 8 cm wide. The fruit is fleshy, less than 3 cm in diameter on wild plants but much larger in cultivated forms [1]. The fruit is botanically classified as a berry and contains numerous small, soft seeds which are edible, but are bitter because they contain an insignificant amount of nicotinic alkaloids [2]. Solanum species extracts have been shown to possess anticancer properties for centuries around the world, including China [3– 5]. However, the underlying mechanisms for their activities remain to be elucidated. The fruits of Solanum incanum Linnaeus are extensively used in Kenya for the treatment of cutaneous mycotic infections and other pathological conditions. The therapeutic activity of the berries has been attributed to their content of solanine and related glycoalkaloids, which are saponins and cytostatic poisons. Though the S. incanum is extensively used for pain and fever management much of the study has centered on it anti-microbial [6, 7] and anti-tumour [8, 9] effects.

**Materials and Methods**

**Sample Collection:**

The Solanum incanum fruits were collected from Kadamboor hills, Sathyamangalam, Erode district, Tamilnadu. This plant was authenticated by Botanical Survey of India, Tamilnadu Agriculture University (TNAU), Coimbatore. The authenticated voucher number is BSI/SRC/5//2/2012-13/Tech.312.

**Preparation of Fruit extract:**

The fruit of Solanum incanum was thoroughly washed in distilled water, cut into small pieces, shade dried and powdered. 20gm of the dried powder were weighed and dispensed in to 100ml of ethanol in conical flask and kept in a shaker for 48 hrs. The extract was again filtered through Whatmann's No.1 filter paper and the ethanol was evaporated and dried extract was used for analysis.

**Preliminary Phytochemical Screening:**

Phytochemical screening of ethanolic extract of Solanum incanum fruit for the presence of these secondary metabolites: Alkaloids (Draggendorff's), Flavonoids, Saponins (Frothing test), Tannins (5% ferric chloride), Terpenoids (2,4-dinitro-phenyl hydrazine), Glycosides (fehling's solution), Steroids (Liebermann's Burchard test) were evaluated according to the methods described by Ayoola G A (2008).

**Thin Layer Chromatography:**

Ethanolic extract of Solanum incanum fruit was subjected to thin layer chromatographic studies, to find out the probable number of compounds present in them.

**Preparation of the plates:**

The adsorbent used for thin layer chromatography was Aluminum pre coated TLC plates. Various Solvent systems were used to detect the phytonutrients such as Alkaloids, Flavonoids, Triterpenoids, Glycosides, Steroids and the bands were visualized under UV light at 366 nm.

**SOLVENT SYSTEMS USED TO DETECT PHYTOCHEMICALS:**

**Detection for alkaloids:**

Toluène: Ethyl acetate: Diethyl amine (7:2:1)
Chloroform: Acetone: Diethyl amine (5:4:1)
Butanol: Chloroform: Diethyl amine (7:2:1)
Chloroform: Ethanol (9: 1)
Toluene: Ethyl acetate: Formic acid (5:4:1)
N-Butanol: Acetic acid: Distilled water (4:1:1)
$CHCl_3$: Methanol: Toluene (4:2:2)

**Detection of Flavonoids:**

$CHCl_3$: Methanol: Toluene (40:5:5)
Methanol: $CHCl_3$ (9:1)
Ethyl acetate: Methanol: Distilled water (55:15: 4)
Toluene: Ethyl acetate (93:7)

**Detection of Terpenoids:**

Toluene: CHCl3: ethanol (40:40:10)
Ethyl acetate: Formic acid: glacial acetic acid: Distilled water (100:11:11:26)
$CHCl_3$: Ethyl acetate (9:1)

**Detection of Glycosides:**

Ethyl acetate: Benzene (2:1)
Benzene: Ethanol (9:1)
$CHCl_3$: Acetic acid (9:1)
Toluene: $CHCl_3$ (9:1)
Detection of Steroid:
Petroleum ether: Ethyl acetate (7:3)

**High Performance Thin Layer Chromatography**

**Procedure:**

The Solanum incanum fruit methanolic extract was centrifuged at 3000rpm for 5min. This solution was used as test solution for HPTLC analysis.2µl of test solution and 2µl of standard solution was loaded as 5mm band length in the 3 x 10 Silica gel $60F_{254}$ TLC plate using Hamilton syringe and CAMAG LINOMAT 5 instrument. The samples loaded plate was kept in TLC twin trough developing chamber (after saturated with Solvent vapor) with respective mobile phase and the plate was developed in the respective mobile phase up to 90mm.The developed plate was dried by hot air to evaporate solvents from the plate. The plate was kept in Photo-documentation chamber (CAMAG REPROSTAR 3) and captured the images at Visible light, UV 254nm and UV366nm.The developed plate was sprayed with respective spray reagent (Alkaloid) and dried at 100°C in Hot air oven. The plate was photo-documented in Visible light and UV 366nm mode using Photo-documentation (CAMAG REPROSTAR 3) chamber. After derivatization, the plate was fixed in scanner stage (CAMAG TLC SCANNER 3) and scanning was

done at visible light 500nm. The Peak table, Peak display and Peak densitogram were noted. The software used was win CATS 1.3.4 version.

**Determination of Solasodine:**
Mobile phase: Ethyl acetate-Methanol-Water (10: 1.35: 1)

Spray reagent: Dragendorff's reagent followed by 10% Ethanolic sulphuric acid reagent.

Detection: Brownish blue, Yellow coloured zone at Visible light mode were present in the tracks, it was observed from the chromatogram after derivatization, which confirmed the Presence of Alkaloid in the given standard and may be in the sample.

**Determination of Quercetin**
**Mobile phase: Toluene-Acetone-Formic acid (4.5: 4.5: 1)**

**Spray reagent: 1% Ethanolic Aluminium chloride reagent.**

**Detection: Yellow, Blue, Yellowish blue coloured fluorescent zone at UV 366nm mode were present in the tracks, it was observed from the chromatogram after derivatization, which confirmed the Presence of Flavonoid in the given standard and may be in the sample.**

**RESULTS AND DISCUSSION**:
The preliminary phytochemical analysis of Solanum incanum fruit shows the presence of Alkaloids, Flavonoids, Carbohydrates, Glycosides, Phenols, Steroids and Proteins. The results were shown in table 1.The TLC procedure was optimized with a view to separate the compounds and to identify various phytochemicals such as Alkaloids, steroids, Terpenoids, Glycosides and flavonoid in the extract. The developed TLC plates were viewed under UV light at 366nm, it shows presence of Alkaloids, steroids, Terpenoids, Glycosides and flavonoid, the numbers of bands, color, Rf value and Solvent system was illustrated in table2 and Figure1.

HPTLC analysis of the ethanol fruit extract of Solanum incanum was carried out along with the standard alkaloid solasodine and standard flavonoid quercetin and Ethyl acetate-Methanol-Water (10: 1.35: 1), : Toluene-Acetone-Formic acid (4.5: 4.5: 1) are the mobile phase respectively. The number of bands obtained was eight alkaloids (Fig.2) and six flavonoids. The identity of the bands of solasodine and quercetin in the ethanol extract was confirmed by comparing the UV-Vis absorption spectra with those of standards using a CAMAG TLC scanner 3 (Fig.3a, 3b, Fig 4a, 4b). The standard solasodine has Rf value of 0.87(Table-3) and quercetin has Rf value 0.60(Table-4).

**CONCLUSION:**
The phytochemical studies showed the presence of most of the biologically active compounds in the fruit. It is generally realized that for monitoring quality, HPTLC fingerprinting is ideal which involves comparison between a standard and a sample. The chromatographic studies conducted with the ethanol fruit extract of Solanum incanum revealed an appreciable amount of alkaloid solasodine and flavonoid quercetin, which confirms its medicinal value.

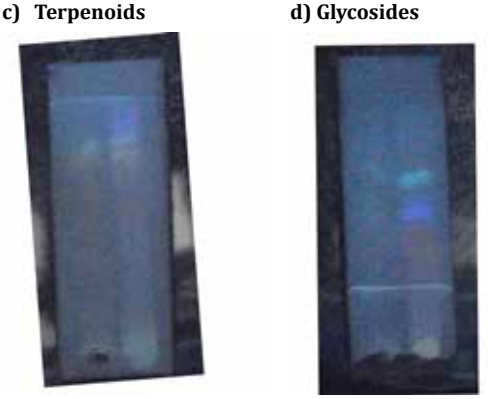
**Table 1: Preliminary Phytochemical analysis of ethanolic extracts of *Solanum incanum* Fruit**

| PHYTOCHEMICAL TEST | ETHANOLIC EXTRACT OF Solanum Incanum Fruit |
|---|---|
| ALKALOIDS:<br>a) Dragendroff's Test<br>b) Wagner's Test<br>c) Meyer's Test | <br>Present<br>Present<br>Present |
| SAPONINS<br>a)Sodium bicarbonate test | <br>Absent |
| CARBOHYDRATES<br>a) Benedict's Test<br>b) Mollisch's Test<br>c) Fehling's Test | <br>Present<br>Present<br>Present |
| PROTEINS<br>a) Millon's Test | <br>Present |
| PHENOLS<br>a) Ferric Chloride Test<br>b) Lead acetate Test<br>c) Liebermann test | <br>Present<br>Present<br>Present |
| STEROIDS<br>a) Libermann Burchard's test<br>b) Salkowski test | <br>Present<br>Present |
| GLYCOSIDES | Absent |
| RESINS | Present |
| TANNINS<br>a) Ferric Chloride Test<br>b) Lead acetate Test | <br>Present<br>Present |
| THIOLS | Absent |

**Figure 1: Thin Layer Chromatography of *Solanumincanum* fruit**

**a) Alkaloids** **b) Steroids**

**c) Terpenoids** **d) Glycosides**



**e) Flavonoids**

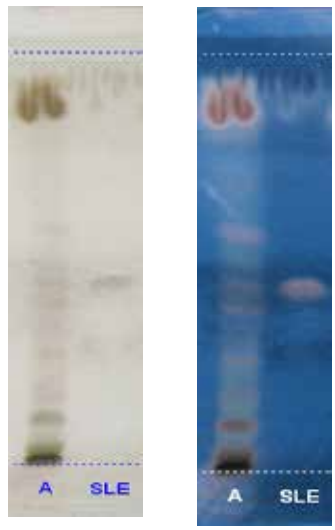
**Table 2: Thin Layer Chromatography of *Solanumincanum* fruit**

| S. No | Phytonutrient | Solvent system | Color of bands Viewed under UV light at 366 nm | $R_f$ Value |
|---|---|---|---|---|
| 1. | Alkaloid | $CHCl_3$: Methanol: Toluene(4:2:2) | Green<br>Pink<br>Yellow | 0.006<br>0.01<br>0.007 |
| 2. | Flavonoids | $CHCl_3$;Methanol: Toluene(33:7:10) | Yellow<br>Blue | 0.02<br>0.03 |
| 3. | Triterpenoids | Toluene: $CHCl_3$ : Ethanol (40:40:10) | Yellow<br>Red<br>Blue | 0.02<br>0.01<br>0.03 |
| 4. | Glycosides | Toluene: $CHCl_3$ (9:1) | Red<br>Blue<br>Blue | 0.06<br>0.03<br>0.03 |
| 5. | Steroids | Petroleum ether: Ethyl acetate (7:3) | Red<br>Blue<br>Blue | 0.03<br>0.02<br>0.05 |

**Table 3: HPTLC results of methanol fruit extract of *Solanumincanum* and the Standard Solasodine**

| Track | Peak | Rf | Height | Area | Assigned substance |
|---|---|---|---|---|---|
| Sample A | 1 | 0.02 | 228.1 | 5793.4 | Unknown |
| Sample A | 2 | 0.11 | 144.8 | 3340.8 | Alkaloid 1 |
| Sample A | 3 | 0.17 | 37.2 | 617.9 | Alkaloid 2 |
| Sample A | 4 | 0.19 | 20.0 | 269.1 | Unknown |
| Sample A | 5 | 0.24 | 18.6 | 190.0 | Unknown |
| Sample A | 6 | 0.27 | 79.2 | 1518.5 | Alkaloid 3 |
| Sample A | 7 | 0.32 | 94.3 | 2576.5 | Unknown |
| Sample A | 8 | 0.34 | 123.7 | 1671.0 | Unknown |
| Sample A | 9 | 0.35 | 135.6 | 2481.6 | Unknown |
| Sample A | 10 | 0.41 | 176.5 | 6716.3 | Alkaloid 4 |
| Sample A | 11 | 0.44 | 178.4 | 2337.9 | Alkaloid 5 |
| Sample A | 12 | 0.45 | 176.5 | 6716.3 | Alkaloid 6 |
| Sample A | 13 | 0.48 | 159.9 | 2134.2 | Alkaloid 7 |
| Sample A | 14 | 0.50 | 162.3 | 2705.3 | Unknown |
| Sample A | 15 | 0.58 | 44.9 | 1060.6 | Alkaloid 8 |
| Sample A | 16 | 0.63 | 25.6 | 291.6 | Unknown |
| Sample A | 17 | 0.65 | 29.1 | 387.1 | Unknown |
| Sample A | 18 | 0.68 | 47.6 | 987.8 | Unknown |
| Sample A | 19 | 0.87 | 588.5 | 56846.8 | Unknown |
| SLE | 1 | 0.44 | 287.2 | 8867.0 | Solasodine standard |

**Figure 2: Identification of Solasodine in *Solanumincanum* by HPTLC**




**Visible light UV 366nm**

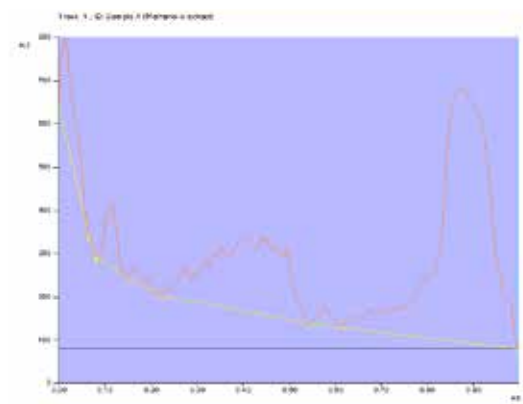


**Figure 3a: Ethanolic extract of Solanum incanum Baseline display (Scanned at 500nm)**

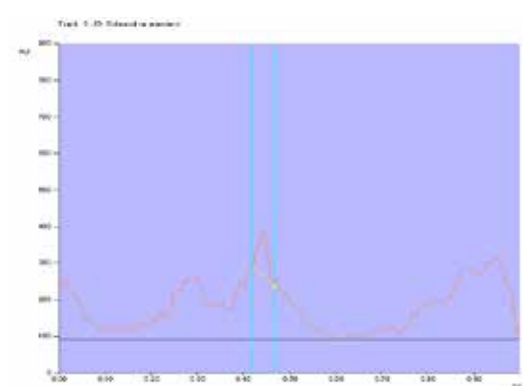


**Figure 3b: Solasodine standard Baseline display (Scanned at 500nm)**

**Table 4: HPTLC results of methanol fruit extract of *Solanumincanum* and the Standard quercetin**

| Track | Peak | Rf Value | Height | Area | Assigned substance |
|---|---|---|---|---|---|
| Sample A | 1 | 0.03 | 59.5 | 965.0 | Flavonoid 1 |
| Sample A | 2 | 0.14 | 43.8 | 1484.0 | Unknown |

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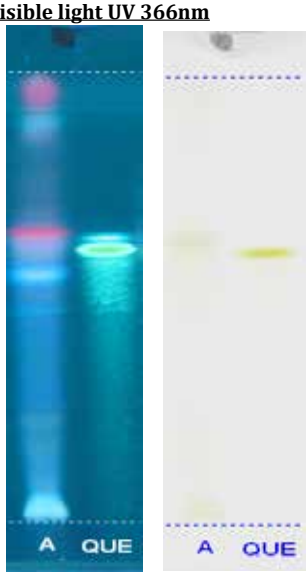
| | | | | | |
|---|---|---|---|---|---|
| Sample A | 3 | 0.20 | 40.2 | 1686.9 | Flavonoid 2 |
| Sample A | 4 | 0.38 | 23.2 | 580.5 | Unknown |
| Sample A | 5 | 0.42 | 38.1 | 936.4 | Unknown |
| Sample A | 6 | 0.46 | 83.8 | 2442.3 | Flavonoid 3 |
| Sample A | 7 | 0.55 | 165.4 | 5641.7 | Flavonoid 4 |
| Sample A | 8 | 0.61 | 328.8 | 22632.4 | Flavonoid 5 |
| Sample A | 9 | 0.72 | 126.1 | 4925.9 | Unknown |
| Sample A | 10 | 0.90 | 206.2 | 8396.2 | Flavonoid 6 |
| Sample A | 11 | 0.96 | 102.9 | 2521.0 | Unknown |
| QUE | 1 | 0.60 | 735.0 | 19045.5 | Quercetin standard |

**Figure 4: Identification of Quercetin in *Solanumincanum* by HPTLC**

**Visible light UV 366nm**



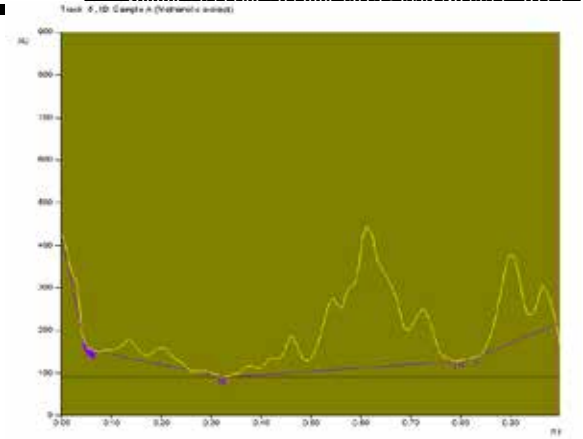



**Figure 5a: Ethanolic extract of Solanum incanum Baseline display (Scanned at 254nm)**

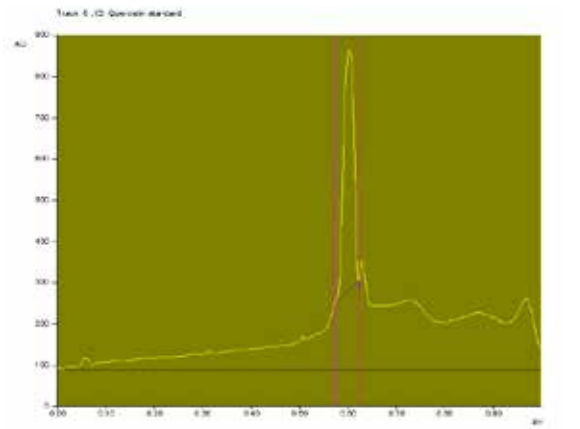


**Figure5b: Quercetin standard Baseline display (Scanned at 254nm)**

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