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Research Article

Morpho-anatomy, phytochemical and HPTLC profiling of *Solanum pubescens* Willd. and *Solanum torvum* Swartz. important medicinal plants used in Siddha System of Medicine

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Abstract

Background: *Solanum pubescens* Willd. and *Solanum torvum* Swartz are important medicinal plants extensively used in the Siddha system of medicine. Due to their therapeutic significance and the possibility of adulteration or substitution, reliable pharmacognostic and phytochemical characterization is essential for the accurate identification, quality assurance, and standardization. Objective: The present study aimed to investigate and compare the morpho-anatomical characteristics, phytochemical constituents, and High-Performance Thin-Layer Chromatography (HPTLC) profiles of *Solanum pubescens* and *Solanum torvum* to establish diagnostic parameters for their authentication and quality evaluation. Methods: Fresh plant materials were collected and subjected to detailed macroscopic and microscopic examinations using standard pharmacognostic procedures. Anatomical studies included transverse sectioning and epidermal studies of leaf samples to identify diagnostic features. Preliminary phytochemical screening was performed to detect major classes of secondary metabolites. HPTLC fingerprinting was carried out using the solvent system *toluene: ethyl acetate: formic acid* (7:3:0.5, v/v/v), and chromatographic profiles were documented under UV light before and visible after derivatization to check characteristic phytochemicals. Results: Both species exhibited distinct morpho-anatomical features that enabled their differentiation. Microscopic examination revealed characteristic variations in trichome, distribution of collenchyma, vascular bundle, calcium oxalate crystals, epidermal cells etc. HPTLC profiling generated fingerprint patterns with multiple characteristic bands and R_f values, demonstrating species-specific chemical profiles useful for authentication and quality control. Conclusion: The combined morpho-anatomical, phytochemical, and HPTLC investigations provide reliable diagnostic parameters for distinguishing *Solanum pubescens* Willd. and *Solanum torvum* Swartz.

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Keywords: *Solanum pubescens*, Micro-morphology, Physico-chemical analysis, Qualitative analysis, HPTLC fingerprinting

Introduction

The Solanaceae (nightshade family) is represented by 98 genera and 2,700 species with great diversity of habitat and morphology. The members of the Solanaceae are erect or climbing, annual or perennial herbs and mostly shrubs (1). The genus *Solanum* L. consists of about 1500 species with wide distribution in the tropical and sub-tropical areas (2). Though the genus is renowned owing to its economical values such as food crops (eg. *Solanum tuberosum* L. (potato), *S. lycopersicum* L. (tomato), *S. melongena* (Mill.) Dunal (egg plant) etc., a number of species of *Solanum* are used as medicine for the treatment of various ailments in Indian Traditional Medicine System such as Ayurveda, Siddha, Unani as well as folklore medicine (eg. *S. aculeatissimum* Jacq. *S. americanum* Mill, *S. torvum* Sw., *S. trilobatum* L etc.) (3, 4).

Solanum pubescens Willd. is an annual erect shrub found abundant in the forest and hilly areas of south Indian states viz. Tamil Nadu, Andhra Pradesh and Kerala. It is commonly known as thorn less turkey berry in English and *Kattusundai, Sundakkai* and *Kathirichundai* in Tamil (5, 6). It has been observed that *S. pubescens is* widely used by the local traditional practitioners for treating various ailments as it commonly available in the vicinity during the medico-ethnobotanical survey conducted since 2022 in

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Tamil Nadu. Previous documentation studies have shown that whole plants are used to treat diseases such as headache, menstrual pain, rheumatoid arthritis, tuberculosis, ulcers and whooping cough (7, 8). The leaves reportedly used in the treatment various skin diseases especially eczema, fruits are used as an anthelmintic also they are being used in local market to substitute fruits of *S. torvum*.

The medicinal importance of the *Solanum* species is owing to the presence of C_{27} – steroidal alkaloids solasodine, a vital precursor for generation of steroid hormones (9). Solasodine was explored in many species of *Solanum* viz. *S. anguivi*, Lam., *S.nigrum* L., *S. melongena* L., *S. virginianum* L., and *S. torvum* Sw. (10) used to treat various ailments such as liver disorders, fever, asthma and hormonal imbalance (11). *S. pubescens* is distinguished from other *Solanum* species by housing a rare alkaloid named *Solanopubamine - Solanopubamides A & B* (12, 13). Still, there is no HPTLC fingerprint data available on *S. pubescens* for delimiting the species among other closely related species in the raw drug market. Further, the pharmacognostical attributes of *S. pubescens* are also yet to be evidently documented as it is not explored thoroughly. In this perspective, the present study aimed to evaluate the morphological, pharmacognostical, phytochemical and HPTLC profiling of leaf samples of *S. pubescens* comparing with *S. torvum* in a view to identify, recognise and authenticate these species in order to avoid the chance of mixing up, adulteration and/or substitution in the raw drug market.

Materials and Methods

Sample collection

The fresh leaf materials of *Solanum pubescens* Willd. and *Solanum torvum* Swartz. were collected from different regions of Salem and Dharmapuri Districts of Tamil Nadu. Samples were processed for herbarium (14) and botanically identified using the regional floras (15-18). Voucher specimens were stored in the herbarium section of Siddha Medicinal Plants Garden and Research Centre, Mettur Dam, Salem District, Tamil Nadu. The healthy leaf samples were thoroughly cleaned and used for further studies.

Morpho- anatomical studies

The transverse sections leaf samples of *S. pubescens* and *S. torvum* were taken by hand using stainless razor blade. The uniformly thin sections were sufficiently stained with 'Safranin O'. The properly stained materials were mounted in glycerine on a clean glass slide and observed under MX 21i LED Trinocular microscope at various magnifications (10x, 20x and 40x) and pictures were captured accordingly and compared with allied species; *S. torvum.*

Physicochemical analysis

Fresh leaves of *S. pubescens* and *S. torvum* were washed in distilled water and shade dried. The dried leaves were pulverized in a mixer, sieved with a fine mesh and used for the analysis of loss of drying, extractive values, total ash, water soluble ash, acid insoluble ash and pH analysis according to standard protocol (19).

Preliminary phytochemical analysis

The leaf powder of *S. pubescens* and *S. torvum* were subjected to consecutive extraction in a Soxhlet apparatus using distilled ethanol. The extract was concentrated under reduced pressure in Rotavapor and further dried in vacuum desiccators. The crude extract was stored in an air tight container for future use. The preliminary phytochemical analysis was carried out using standard procedure (20-22).

HPTLC

Sample Preparation

One gram of powdered leaf samples were sonicated with 10 ml ethanol for 10 minutes. The extract were filtered and taken in sample vials.

Methodology

The HPTLC was performed on silica coated TLC plate (60F254) in Twin trough chamber (CAMAG) (10×10 cm) pre-saturated with the mobile phase, Toluene: Ethyl acetate: Formic acid (7:3:0.5) v/v/v. Samples, 20 µl and 25 µl of the extract was applied using Camag's Linomat 5 applicator. The plate was developed up to 85 mm from the bottom and then photo documented using Camag's TLC Visualizer under UV λ254 nm and UV λ366 nm followed by scanning using Camag's scanner 4 at λ 254 nm (D2 lamp, Absorption mode) and finger print profiles were retrieved. The plate was derivatized with vanillin sulphuric acid, heated at 105°C and scanned at 520 nm (W lamp, Absorption mode).

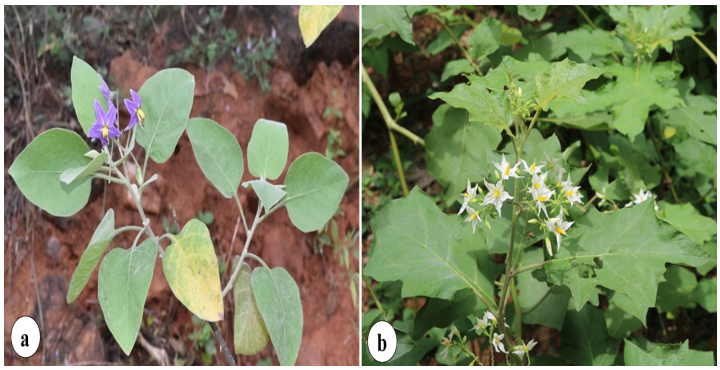
Results

Morphological analysis

Solanum pubescens is a shrub, 2(3) m; branchlets, fulvous-pubescent. Leaves deltoid to elliptic- ovate, 5.5-12× 3.5-6.5 cm, chartaceous, lateral nerves ca. 8, base obliquely truncate to (sub) cordate, apex acute; petiole to 5 cm. Racemes axillary; peduncle to 4 cm; pedicel to 2 cm, Calyx - lobes lanceolate, 7mm, herbaceous, glabrous within, shortly decurrently, acuminate. Corolla purple to violet, 2.5 cm across; lobes 5, oblanceolate, 1 cm, stellate- pubescent along nerves within, acuminate. Stamens 5; filaments 2 mm; anthers linear – oblong, 8 mm. Ovary 1.5 × 1 mm; style 1.2 cm; stigma capitate. Berry 1.5 cm across; seeds minutely scaly (Figure 1a) (15, 17).

Solanum torvum is an armed shrub, 2.5(4) m; prickles scattered. Leaves sinuate, 8-15 × 6-12 cm, chartaceous, lateral nerves ca. 5 pairs, sparsely stellate –pubescent above, base obliquely subcordate to truncate, apex acute; petiole to 6 cm. Corymbose cyme extra-axillary; peduncle to 5 cm; pedicel to 1 cm. Calyx copular; lobes oblong, 4 mm, basally connate, pubescent without, acuminate. Corolla white, 2.5 cm across; lobes 5, oblong, 1.5 cm, stellate-pubescent without, acute. Stamens 5 filaments 1.5 mm; anthers 7mm. Ovary 1.5 mm; style 8.5 mm; stigma obtuse. Berry globose, 1 cm across; seeds circular, smooth (Figure 1b) (15, 17).

Figure 1: Habit; (a) *Solanum pubescens* and (b) *Solanum torvum*



Anatomical analysis

Petiole

The transverse section of petiole of *Solanum pubescens* (Fig. 2a) is plano-convex in outline and a narrow-winged projection on the upper side. The epidermis is single layered with sparse stellate trichomes. The 7-10 layered angular collenchymatous hypodermis is followed by parenchymatous ground tissue with sparse idioblasts of crystal sand. The vascular bundles are bicollateral, similar to the pattern found in the lamina. In *Solanum torvum,* transverse section of petiole (Fig. 2d) is circular in outline and the epidermal layer consists of plenty of multicellular, stellate trichomes. Hypodermis composed of 3-4 layers of collenchyma cells and a wide parenchymatous ground tissue occupied the central portion. The ground tissue is embedded with an arc of centrally located bicollateral vascular bundle and microsphenoidal crystals of calcium oxalate

Midrib & Lamina

The epidermis of *Solanum pubescens* consists of single layered uniseriate parenchyma cells covered by thin cuticle. Numerous multicellular, stellate trichomes and multicellular glandular trichomes were present. Beneath the epidermis, hypodermis with 4-7 layers of angular collenchyma cells were present, followed by the ground parenchyma tissue. The vascular bundles are conjoint and bicollateral, and composed of only one central bundle, in arc shape on apical portion, which is "U" shaped toward the median to basal portions. The mesophyll is dorsoventrally arranged with 4-5 layers of uniseriate palisade tissues in the adaxial side and 4-5 layers of spongy parenchyma with sparse idioblasts of crystal sand in the abaxial side. The crystals are also observed in the external phloem. Xylem cells are lignified. (Fig 2: b & c). In *Solanum torvum*, epidermal cells are single layered covered with cuticle and multicellular stellate trichomes and few multicellular glandular trichomes. Underneath the epidermis 6-8 layers of collenchymatous hypodermal layer followed by ground parenchyma cells with microsphenoidal crystals of calcium oxalate were present. A central arc shaped conjoint bicollateral vascular bundle more or less meristelic appearance was present embedded in the ground tissue. The dorsiventral mesophyll is similar to that of *Solanum pubescens* with single layer of palisade parenchyma and 4-5 rows of spongy parenchyma cells. (Fig. 2: e & f).

Epidermis and Stomata

The cells of upper and lower epidermal layers of *Solanum pubescens* are sinuous and wavy in nature and covered by thin cuticle. The cells of upper epidermis are comparatively larger than that of lower. The upper and lower epidermis has anisocytic type stomata. Glandular, non- glandular and stellate trichomes occur on both surfaces and they are sessile with reduced midpoint (Fig. 3: a-c), In *Solanum torvum,* the upper epidermal cells have sinuous anticlinal walls and that of lower epidermis is slightly waved in nature. Both upper and lower epidermis has anisocytic and anomocytic type of stomata. Porrect stellate trichomes occurs on both the surfaces and are stalked or sessile with reduced midpoint (Fig. 3: d-f).

Physico-chemical Analysis

Results of physico-chemical parameters of *S. pubescens* and *S. torvum* are summarized in Table 1 which shows 10.32 % and 11.25 % of moisture content respectively. The total ash of S. *pubescens* was estimated as 11.43 % which is higher than *S. torvum*'s ash value of 9.21%. The water-soluble ash was 6.32 % and 6.19% respectively which may be considered as same. The acid insoluble ash values were calculated as 0.56 % and 0.20 % respectively. Alcohol soluble extractive value of *S. torvum* (38.20%) is higher than that of *S. pubescens* (23.09 %). Whereas the water-soluble extractive value of

Figure 2: Transverse section of Petiole, Leaf Midrib and Lamina of *S. pubescens* (a-c) and *S. torvum* (d-f); Tr. Trichome, Gt: Ground Tissue, Vb: Vascular bundle, PP- palisade parenchyma, SP- spongy parenchyma.

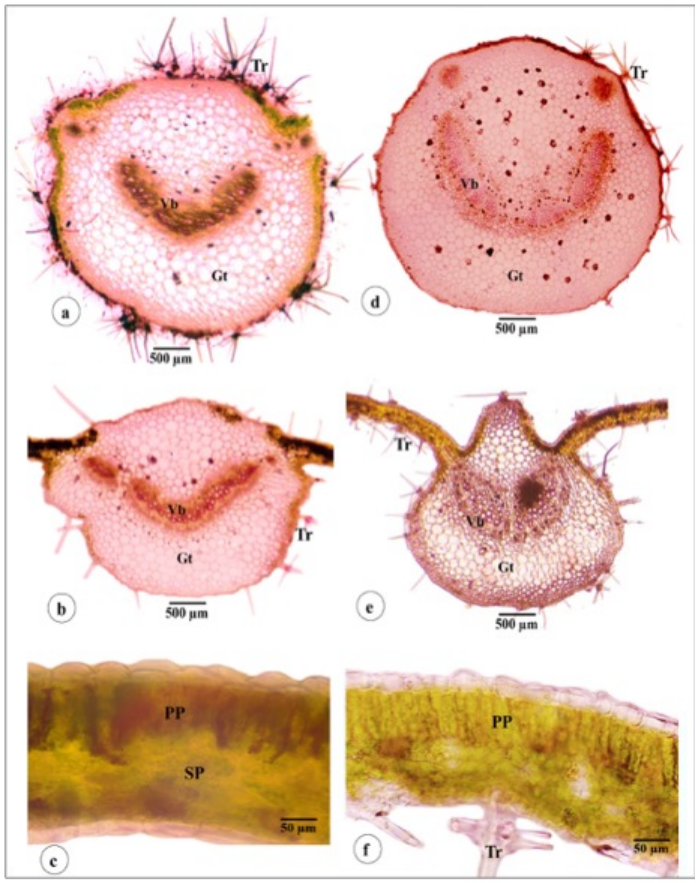
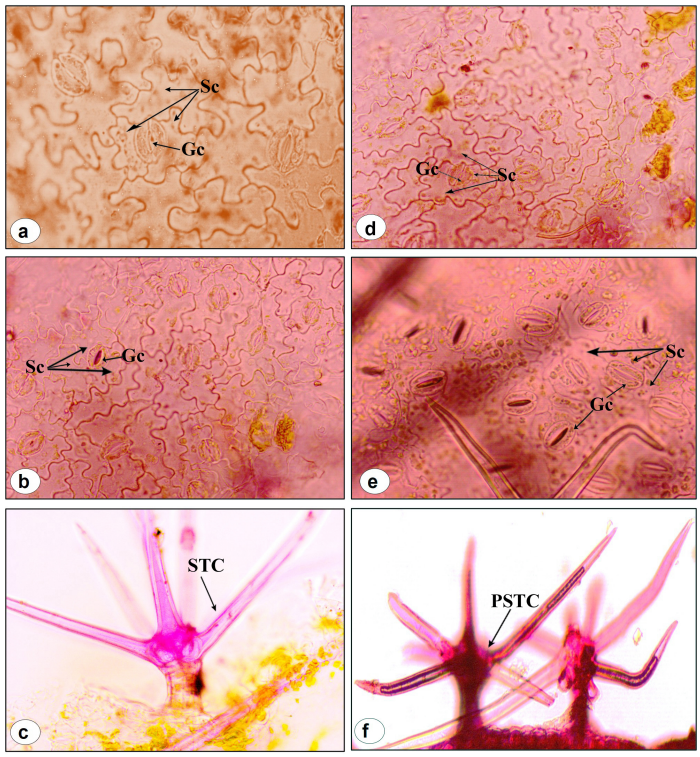


Figure 3: Epidermal study; (a-c): *S. pubescens* and (d-f): *S. torvum;* a & d: Upper epidermis, b & e: lower epidermis, Gc: Guard cell of stomata, Sc: Subsidiary cell, STR- stellate trichome, PSTR-Porrect stellate trichome.



S. torvum (21.15 %) is less than *S. pubescens* (28.37%) The pH value of *S. pubescens* is acidic (5.55) while *S. torvum* is towards neutral (6.53).

Phytochemical Analysis

Qualitative phytochemical analysis of leaves of *Solanum pubescens* and *Solanum torvum* revealed the presence of medicinally important bioactive compounds. The preliminary phytochemical screening results of ethanolic leaf extracts have been detailed in the Table-2 which shows the presence of important groups of secondary metabolites viz., alkaloids, phenols, tannins, triterpenoids and carbohydrates.

Table 1: Physico-chemical analysis of leaves of *Solanum species*

S.No.	Parameter	Mean value ± SD	
		S. pubescens	***S. torvum***
1	Moisture content (%)	10.32±0.09	11.25±0.21
2	Total ash (%)	11.43±0.29	9.21±0.36
3	Water soluble ash (%)	6.32±0.81	6.19±0.25
4	Acid insoluble ash (%)	0.56±0.79	0.20±0.02
5	Alcohol soluble extractive (%)	23.19±0.55	38.20±1.22
6	Water soluble extractive (%)	28.37±1.06	21.15±0.96
7	pH (10% solution)	5.55±0.21	6.53±0.11

Table 2: Phytochemical screening of leaves of *Solanum species*

S.No	Phytochemical	Test	S. *pubescens*	*S. torvum*
1	Alkaloids	a. Mayer's reagent b. Wagner's reagent c. Hager's reagent	+ + +	+ + +
2	Protein	a. Biuret's test	-	-
3	Carbohydrates	a. Benedict's test b. Fehling's test	+ +	+ +
4	Glycosides	a. Borntrager's test b. Anthrone sulphuric acid test c. Keller-Killani test	- + -	- + -
5	Fixed oil and Fats	a. Saponification test	-	-
6	Phenols	a. Ferric chloride test b. Iodine test c. Nitric acid test	+ + +	+ + +
7	Tannins	a. Lead acetate test b. Gelatin test	+ +	+ +
8	Flavonoids	a. Shinoda's test	+	-
9	Phytosterols	a. Libermann- Burchard's test	+	+
10	Triterpenoids	a. Noller's test	+	+

HPTLC

The HPTLC profiling revealed a more enlightened details of phyto-constituents present in the leaf samples of *S. torvum* and *S. pubescens*. The visualization of the plate after the development of the chromatogram showed the presence of 9 bands in *S. torvum* and 10 spots in *S. pubescens* at UV λ254 nm. The common spots appeared at R_f 0.23, 0.32, 0.37, 0.40, 0.48, 0.76 and 0.93. Totally 12 spots appeared for *S. torvum* and 10 spots appeared for *S. pubescens* at UV λ366. The common spots appeared at R_f 0.18 (white color), 0.26, 0.35, 0.40, 0.50, 0.54, 0.63, 0.72 (all red color) and 0.81 (blue color). The visualization of the plate in white light after derivitization has given more clear coloured visible bands 11 nos for *S. torvum* and 10 nos for *S. pubescens*. The bands at R_f 0.16, 0.27. 0.33, 0.61, 0.66, 0.72, 0.78 and 0.92 appeared common to both species (Table 3 & Fig. 4). Similarly, the scanning result of the plate at UV λ 254 nm exhibited 11 peaks for *S. torvum* and 12 peaks for *S. pubescens*; while the derivatized, plate has produced 19 peaks for *S. torvum* and 18 peaks for *S. pubescens* (Fig.5 a & b).

Table 3. R_f value of the bands visulaization under λ=254 nm, λ=366 nm and white light

UV 254 nm				UV 366 nm				White light after spray with Vanillin sulphuric acid			
S. torvum		***S. pubescens***		***S. torvum***		***S. pubescens***		***S. torvum***		***S. pubescens***	
R_f	Color	R_f	Color	R_f	Color	R_f	Color	R_f	Color	R_f	Color
-	-	0.16	Green	0.18	White	0.18	White	0.16	Purple	0.16	Purple
0.23	Green	0.23	Green	0.26	Red	0.26	Red	0.27	Purple	0.27	Purple
0.32	Green	0.32	Green	0.30	Red	-	-	0.33	Green	0.33	Purple
0.37	Green	0.37	Green	0.35	Red	0.35	Red	-	-	0.42	Yellow
0.40	Green	0.40	Green	0.40	Red	0.40	Red	0.50	Green	-	-
0.48	Green	0.48	Green	-	-	0.43	Red	0.54	Purple	-	-
0.54	Green	-	-	0.50	Red	0.50	Red	-	-	0.58	Yellow
-	-	0.58	Green	0.54	Red	0.54	Red	0.61	Purple	0.62	Yellow
0.62	Green	-	-	0.63	Red	0.63	Red	0.66	Blue	0.66	Blue
-	-	0.65	Green	0.68	Red	-	-	0.72	Blue	0.72	Blue
0.76	Green	0.76	Green	0.72	Red	0.71	Red	0.78	Blue	0.78	Yellow
0.93	Green	0.94	Green	0.76	Red	-	-	0.92	Blue	0.92	Blue
				0.81	Blue	0.81	Blue	0.98	Blue	-	-

Figure 4: TLC plate visualized under λ=254 nm, λ=366 nm and white light

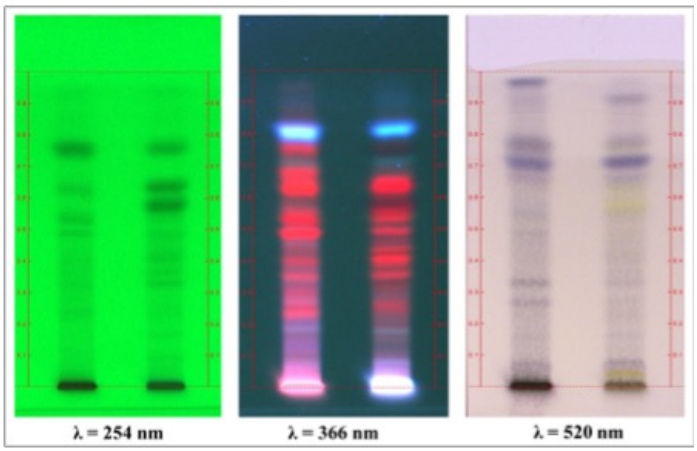
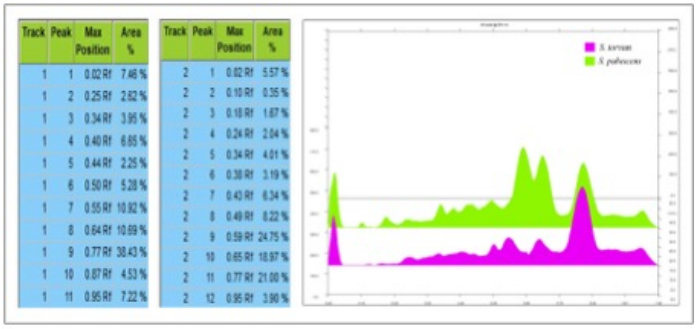


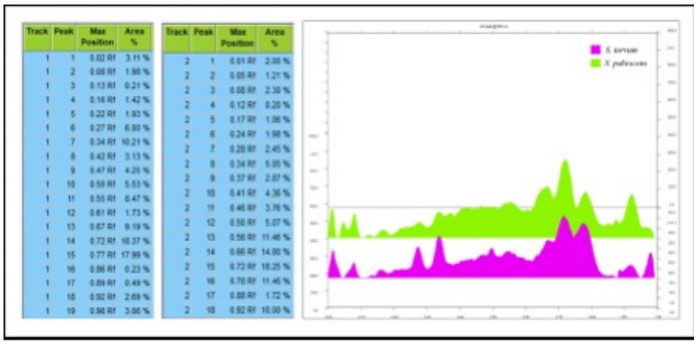
Figure 5a: Peak table & chromatogram of *S. torvum* (Track 1) & *S. pubescens* (Track 2) at λ=254 nm



Track	Peak	Max Position	Area %
1	1	0.02 Rf	7.46 %
1	2	0.25 Rf	2.82 %
1	3	0.34 Rf	3.95 %
1	4	0.40 Rf	6.85 %
1	5	0.44 Rf	2.25 %
1	6	0.50 Rf	5.28 %
1	7	0.55 Rf	10.92 %
1	8	0.64 Rf	10.69 %
1	9	0.77 Rf	38.43 %
1	10	0.87 Rf	4.53 %
1	11	0.95 Rf	7.22 %

Track	Peak	Max Position	Area %
2	1	0.02 Rf	5.57 %
2	2	0.10 Rf	0.35 %
2	3	0.18 Rf	1.67 %
2	4	0.24 Rf	2.04 %
2	5	0.34 Rf	4.01 %
2	6	0.38 Rf	3.19 %
2	7	0.43 Rf	6.34 %
2	8	0.49 Rf	8.22 %
2	9	0.59 Rf	24.75 %
2	10	0.65 Rf	18.97 %
2	11	0.77 Rf	21.00 %
2	12	0.95 Rf	3.90 %

Figure 5b: Peak table & chromatogram of *S. torvum* (Track 1) & *S. pubescens* (Track 2) at λ=520 nm



Track	Peak	Max Position	Area %
1	1	0.02 Rf	3.11 %
1	2	0.08 Rf	1.98 %
1	3	0.13 Rf	0.21 %
1	4	0.16 Rf	1.42 %
1	5	0.22 Rf	1.93 %
1	6	0.27 Rf	6.00 %
1	7	0.34 Rf	10.21 %
1	8	0.42 Rf	3.13 %
1	9	0.47 Rf	4.25 %
1	10	0.50 Rf	5.53 %
1	11	0.55 Rf	8.47 %
1	12	0.61 Rf	1.73 %
1	13	0.67 Rf	9.19 %
1	14	0.72 Rf	16.37 %
1	15	0.77 Rf	17.99 %
1	16	0.86 Rf	0.23 %
1	17	0.89 Rf	0.49 %
1	18	0.92 Rf	2.69 %
1	19	0.96 Rf	3.06 %

Track	Peak	Max Position	Area %
2	1	0.01 Rf	2.00 %
2	2	0.05 Rf	1.21 %
2	3	0.08 Rf	2.30 %
2	4	0.12 Rf	0.20 %
2	5	0.17 Rf	1.06 %
2	6	0.24 Rf	1.98 %
2	7	0.28 Rf	2.45 %
2	8	0.34 Rf	5.05 %
2	9	0.37 Rf	2.07 %
2	10	0.41 Rf	4.36 %
2	11	0.46 Rf	3.76 %
2	12	0.50 Rf	5.07 %
2	13	0.56 Rf	11.46 %
2	14	0.66 Rf	14.00 %
2	15	0.72 Rf	18.25 %
2	16	0.76 Rf	11.46 %
2	17	0.88 Rf	1.72 %
2	18	0.92 Rf	10.00 %

Discussion

Solanum pubescens is known to be used by the local traditional healers for curing diseases as mentioned earlier. The *Solanum torvum* is reported to have therapeutic potential to treat various ailments especially in Siddha and Ayurveda. *Solanum pubescens* and *Solanum torvum* are clearly distinguishable from each other as they possess wide range of variation in morphological attributes especially in flower color, presence and absence of prickles, leaf shape and size etc., the fruits of the *Solanum pubescens* are selling in local market mixed with fruits of *Solanum torvum* and sometimes exclusively in place of it. Moreover, the dried and crushed form of these plants become difficult to distinguish from each other based on the morphology especially in the raw drug market. In such cases, the anatomical, pharmacognostic, physicochemical and phytochemical analyses and HPTLC profiling provide promising information leading to the precise identification of species and it is prime important in the medicine preparation. As there are no such demarcating details available as of now for the *Solanum pubescens,* the present study executed to provide relevant referential data to identify the *Solanum pubescens* from other allied species especially from *Solanum torvum* and thus to avoid the chance of misapplication of the raw drugs in medicines.

In many aspects, the anatomy of *S. pubescens* leaf and petiole are found to be similar with *S. torvum* (23) such as the presence of stellate trichomes, dorsiventral mesophyll, angular collenchyma, anisocytic type of stomata etc. and those are also reported from other species of Solanum (24-27). The leaf anatomy of *S. pubescens* can be distinguished from that of *S. torvum* based on the characteristics of the epidermis, trichomes, and idioblast cells. The presence of stellate glandular trichomes in *S. pubescens* is a characteristic feature of the Solanaceae family, whereas *S. torvum* is characterized by porrect-stellate trichomes that are either sessile or stalked (23). The occurrence of crystal sand-containing idioblasts in the mesophyll of *S. pubescens* is a common feature reported in *Solanum* species (28). In contrast, *S. torvum* possesses idioblasts containing druse crystals, a comparatively rare anatomical feature. Thus, differences in epidermal anatomy, trichome morphology, and the type of crystal-bearing idioblasts serve as reliable diagnostic characters for distinguishing *S. pubescens* from *S. torvum*.

The analysis of physicochemical attribute is another important parameter in determination of adulterants and substitutes. In this study, the physicochemical attributes of *S. pubescens* leaf powder have given significant data (Table 2). The higher water-soluble extractive value of *S. pubescens* reveals the presence of high polar compounds while high value of alcohol soluble extractive value of *S. torvum* reveals the presence of non polar and medium polar compounds. The higher ash value of *S. pubescens* contributes to the acidic pH value. Whereas the comparatively less ash content of *S. torvum* contributes the less acidity of *S. torvum*. pH value (5.55) indicates the acidic nature of the drug which in turn is the indication of dominance of acidic phytosterols over alkaloids (29). Ash values are significant in analyzing the identity and purity of crude drugs especially in the powder form (30).

The preliminary phytochemical screening of ethanolic leaf extract of *S. pubescens* and *S. torvum* detected the presence of important groups of secondary metabolites including alkaloids, phenols, tannins, phytosterols and triterpenoids. But flavonoid was detected only in *S. pubescens*. The presence of alkaloids, glycosides, saponins, phenolic compounds, tannins, flavonoids have already been reported in the methanolic leaf extract of *S. pubescens* (31). Nine known compounds viz., neochlorogenin 6-O-β-D-quinovopyranoside, neochlorogenin 6-O-β-D-xylopyranosyl-(1→3)β-D-quinovopyranoside, neochlorogenin 6-O-α-L-rhamnopyranosyl-(1→3)-β-D-quinovopyranoside (3), solagenin 6-O-β-D-quinovopyranoside, solagenin 6-O-α-L-rhamnopyranosyl-(1→3)-β-D-quinovopyranoside, isoquercetin, rutin, kaempferol and quercetin have been reported from the aerial parts of *S. torvum* (32).

In the present TLC study, few spots were identified as distinguishing spots. Under UV 254 nm, the spots at 0.54 and 0.62 were specific to *S. torvum*; whereas the spots at R_f 0.16, 0.58 and 0.65 were specific to *S. pubescens*. Under UV 366 nm, the spots at R_f 0.30, 0.68 and 0.76 were specific to *S. torvum* and the

spot at R_f 0.43 is specific to *S. pubescens*. The derivatized plate also demonstrated few distinguishing spots at R_f 0.50, 0.54 and 0.93 (specific to *S. torvum*); 0.42 and 0.58 (both yellow specific to *S. pubescens*). *S. torvum* did not show any yellow-colored spots on derivatization with vanillin sulphuric acid revealing the absence of quercetin, isoqercetin, kaempferol and rutin which were already reported in the aerial parts. Whereas, *S. pubescens* showed yellow-colored spots at R_f 0.42, 0.58, 0.62 and 0.78 indicating the presence of flavonoids (33) and navigating the scope for phytochemical isolation work to the researchers (34).

HPTLC fingerprinting can be taken as a basic reliable tool for the authentication, standardization and identification of drugs in quality control from their adulterants and substitutes. HPTLC profiling *S. pubescens* leaf extract gave clear picture on the composition of phytochemicals contained in them where the number of bands and peaks corresponding to different R_f values are the clear indication of the number of particular phytochemical present in them. From the data table of Fig. 5a & 5b, it has been observed that the finger print profile at 254 nm forecasted 11 and 12 peaks respectively in *S. torvum* and *S. pubescens.* This finger print profile revealed two common spots at R_f 0.34 and 0.77. The peaks at R_f 0.77 (38.43%), 0.64 (10.69%) and 0.55 (10.92%) are the major peaks in *S. torvum* while the peaks at R_f 0.59 (24.75%), 0.77 (21 %) and 0.65 (18.97%) are the major peaks in *S. pubescens.* The finger print profile of post derivatized plate at 520 nm, there 19 peaks and 18 peaks were generated respectively in *S. torvum* and *S. pubescens* in which peaks at R_f 0.34, 0.50, 0.72 and 0.92 are common in both. The peak at R_f 0.72 (18.37%) is the major peak in *S. torvum* followed by 0.77 (17.99%), 0.27 (10.21%) and 0.67 (9.19%). Similarly major peaks of *S. pubescens* are 0.72 (18.25%), 0.66 (14.80%), 0.56 (11.46%), 0.78 (11.46%) and 0.92 (10%). Previous study reported the quantification of solanopubamine from the ethanol extract of leaf samples of *S. schimperianum* through HPTLC using the mobile phase *chloroform: methanol: ammonium hydroxide* in the ratio 30:20:1.5 v/v (35,36). The solasodine was reported in the HPTLC analysis of fruits of *S. virginianum* (37,38), *S. nigrum* (39) and *S. torvum* (40). The HPTLC fingerprint pattern obtained in the present study using the solvent system, *toluene: ethyl acetate: formic acid* (7:3:0.5, *v/v/v*) in the ethanolic extract may be taken as a reference to identify the leaf sample of *S. pubescens* and *S. torvum* to avoid chance of adulteration and substitution.

Conclusion

Even though the therapeutic potentials of *S. pubescens* have not yet been well explored evidently, the local traditional healers use this plant for treating various ailments from their experience (unpublished data). The present study aimed to provide demarcating data evidently through the anatomical, pharmacognostical, physico chemical, phytochemical analyses and HPTLC fingerprint profiling to identify and delimit the samples of *S. pubescens* in the raw drug industry thus to keep the integrity and efficacy of medicines by avoiding the chance of adulteration and substitution with other allied species especially with *S. torvum.* The results of the study provided remarkable and potential data in the above perspective and which can be used as a gateway for more engrossed research in future identification of therapeutic compounds of this plant.

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