TLC and HPTLC Fingerprints of Various Secondary Metabolites in the Stem of the Traditional Medicinal Climber, *Solena amplexicaulis*

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Aim of this study was to develop a TLC and a HPTLC fingerprint profiles for various secondary metabolites of methanol extracts of the stem of the traditional medicinal climber, *Solena amplexicaulis*. These studies were carried out as per the methods of Harborne and Wagner *et al.* The profiles of various individual secondary metabolites were made and developed for authentication. The methanol extract of the stem showed the presence of 6 alkaloids, 6 flavonoids, 2 glycosides, 9 saponins and 3 terpenoids. Owing to the presence of rich variety of secondary metabolites, the stem extract of *S. amplexicaulis* is expected to exhibit therapeutic properties.

Key words: *Solena amplexicaulis,* TLC profile, HPTLC profile, bioactive compounds

The current scenario appears to demand for plant drugs throughout the world because of their safety and efficacy[1]. Now a days folklore medicine is being reevaluated by extensive research on different plant species and their therapeutic principles. Chromatographic and spectral fingerprint analysis plays an important role in the quality control of complex herbal medicines[2]. Thin layer chromatography (TLC) is the preliminary step to identify the phytochemical constituents in a sample. High performance thin layer chromatography (HPTLC) can provide an electronic image of the chromatographic fingerprint and a densitogram to detect the presence of marker compounds in a plant sample. Both the methods are efficient, faster, reliable and reproducible[3].

Solena amplexicaulis, commonly called as the creeping cucumber, belongs to the family Cucurbitaceae. The medicinal uses of this species are manyfold[4]. Traditional healers prescribed the tubers of this species as astringent, appetizer, carminative, cardiotonic, digestive, diuretic, expectorant, invigorating, purgative, stimulant, sour and termogenic[5,6]. The whole plant is a potential source of natural antioxidant[7,8], antidiabetic[9] and antibacterial[10] agents. The leaves have good antiinflammatory property, the species is recognized as CNS active, diuretic, febrifuge and hypothermic[6,11]. Crude leaf juice is used to cure jaundice[12]. Raw unripe fruits are eaten to strengthen the body[13]. The decoction of the root is taken orally to cure stomach ache[14], while the seeds are purgative[6].

It is a well known fact that species of medicinal importance contain a rich variety of secondary metabolites, some of which are responsible for the biological activity. To confirm this view, TLC and HPTLC studies were undertaken to explore the various secondary metabolites present in the stems of *S. amplexicaulis*.

The stems of *S. amplexicaulis* were collected separately from the thorny scrub jungles of Madukkarai, Coimbatore district, Tamil Nadu, India. The authenticity of the plant was confirmed by comparing with the reference specimens (vide no: CPS 313) preserved at Botanical Survey of India, Southern Circle, Coimbatore. They were washed thoroughly in tap water, shade-dried, homogenized to a fine powder and stored in air tight bottles. Fifty grams of the powdered stem of *S. amplexicaulis* was extracted with 250 ml methanol at a temperature between 60 and 65° for 24 h using a Soxhlet extractor. The solvent was evaporated in a rotary vacuum evaporator to obtain a viscous semi-solid mass. This semi-dry crude methanol extract was subjected to TLC and HPTLC analysis. TLC and HPTLC studies were carried out using the methods of Harbone[15] and Wagner *et al.*[16], respectively.

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For separating different phytochemical compounds in the methanol extract of the stems of *S. amplexicaulis,* the extract was spotted manually using a capillary tube on pre coated silica gel G TLC plates (15×5 cm, 3 mm thickness). The spotted plates were developed in different solvent systems to detect a suitable mobile phase as per the method of Wagner *et al.*[16,17]. After the separation of phytochemical constituents, reagents such as Dragendorff, 10% sulphuric acid in ethanol, 10% sulphuric acid, 5% ferric chloride, Kedde, vanillin phosphoric acid and vanillin sulphuric acid were sprayed to identify the respective compounds. The colours of the spots were noted and Rf values were calculated.

One hundred milligrams of the methanol extract was dissolved in 1 ml of HPTLC grade methanol and centrifuged at 3000 rpm for 5 min. This solution was used as test solution for HPTLC analysis. Different solvent systems were used to develop HPTLC fingerprint profile for different secondary metabolite groups such as alkaloids, flavonoids, glycosides, terpenoids and saponins[15,16,18]. Two microliters of the sample and 3 µl of standard solution were loaded as 5 mm band length separately on pre coated silica gel $60F_{254}$ aluminum sheets (3×10 cm) using a Hamilton syringe with the help of Linomat 5 applicator attached to a Camag HPTLC system, which was programmed through WIN CATS software. After the application of spots, the chromatogram was developed in twin trough glass chamber (20×10 cm) pre saturated with respective mobile phase. The air-dried plates were kept in a photo documentation chamber (Camag Reprostar 3) and images were captured at visible light, UV 366 nm and UV 254 nm. The chromatograms were scanned by a densitometer at 405 nm after spraying with respective spray reagents and dried at 100° in a hot air oven. The peak number with its height, area and Rf values of fingerprint data were recorded by WIN CATS (1.3.4 version) software.

The current study was taken up to screen the methanol extract of the stem of *S. amplexicaulis* for secondary metabolites and develop fingerprints using TLC and HPTLC techniques. Methanol extract of the stem was subjected to TLC in which different mobile phases were tried in order to separate the bioactive compounds like alkaloids, flavonoids, glycosides, terpenoids and saponins (Table 1). The study revealed the development of one orange or brown coloured band for alkaloids in 2 different solvent systems, one yellow or grey coloured band for flavonoids in 2 different solvent compositions and one distinct band for glycosides. In addition, two violet blue coloured spots for saponins in a single solvent system and two blue coloured spots for terpenoids in 2 different compositions were developed by applying respective spraying reagents. Based on the colour, the secondary metabolites were differentiated and Rf values were calculated. The study revealed that relatively high polarity solvents like chloroform, ethyl acetate and methanol were more suited as mobile phases for the separation of bioactive compounds in the stem of *S. amplexicaulis.*

TABLE 1: TLC SCREENING OF PHYTOCHEMICALS IN METHANOL EXTRACT OF THE STEM OF *SOLENA AMPLEXICAULIS*

Mobile phase	Spraying reagent	Colour of the spot/band	Rf	Compound
Chloroform-methanol				
[3:1.3]	Dragendorff reagent/10% H_2SO_4 in ethanol reagent	Orange/Brown	0.21	Alkaloids
[3:2]			0.25	
Ethyl acetate-methanol-water-glacial acetic acid	10% H_2SO_4/5% ferric chloride solution	Yellow/Grey		Flavonoids
[1.35:0.5:0.5:0.05]			0.91	
Ethyl acetate-methanol-water-toluene				
[1.4:0.5:0.5:0.05]			0.92	
Ethylacetate-methanol	Kedde reagent	Distinct band formation		Glycosides
[1.3:0.5]			0.38	
Chloroform-methanol	Vanillin H_2SO_4 reagent	Violet blue	0.69; 0.55	Saponins
[1.2:0.2]			(2 spots)	
Petroleum ether-ethyl acetate	Vanillin phosphoric acid reagent	Blue	0.39; 0.58	Terpenoids
[2:0.5]			(2 spots)	
Hexane-ethyl acetate			0.55; 0.72	
[1.5:0.5]			(2 spots)	

HPTLC profile of methanol extract of the stem was generated in solvent systems of different polarities in order to ascertain the total number of chemical moieties, which will also help in designing the method of isolation and characterization of bioactive compounds (Table 2).

HPTLC alkaloid profile of methanol extract of the *S. amplexicaulis* stem was presented in Tables 2 and 3 and fig. 1a. Bright orange colour zone at visible light mode was observed in the chromatogram after derivatization, which confirmed the presence of alkaloids in the sample as in the standard. Thirteen compounds were separated and of which 6 were alkaloids at the Rf in the range of 0.18-0.59. The highest peak area was 11283.1 AU and that of the lower one was 1424.5 AU, which were observed at Rf of 0.40 and 0.59, respectively. It is not uncommon to find alkaloids since more than 10 000 different alkaloids have been identified in species from over 300 plant families[19].

Six different types of flavonoids were observed out of 11 bands in the methanol extract of *S. amplexicaulis*. The Rf values determined for the flavonoids were in the range of 0.02-0.64 (Tables 2 and 4 and fig. 1b). The suitable solvent system evaluated was toluene-acetone-formic acid (4.5:4.5:1). Yellow or yellowish blue coloured fluorescence zone at UV 366 nm mode confirmed the presence of flavonoids in the sample and standard. The highest peak area was 32643.4 AU and that of the lowest one was 427.9 AU, which were observed at Rf of 0.21 and 0.55, respectively. More than 6000 different flavonoids have been identified to occur and many of them are responsible for the attractive colors of flowers, fruits and leaves[20]. Biological activities exhibited by some of these flavonoids have resulted in intensive research efforts to understand the impact of these compounds on human health[21].

After derivatization, the pinkish violet colour confirmed the presence of glycosides in the given samples and standard. The sample revealed 12 spots and among them only 2 bands were for glycosides in the Rf level of 0.69 and 0.82 (Tables 2 and 5 and fig. 1c). Reliable solvent system to observe the above separation is ethylacetate: ethanol: water (8:2:1.2). Many plant glycosides are reported to have useful medicinal properties. In animals and humans, poisons are often eliminated from the body as glycosides[22].

The HPTLC chromatogram can be best observed under daylight, UV 254 nm and 366 nm before and after derivatization. Nine bands of different types of saponins were seen before derivatization at visible mode. Due to the great variability of their structures, saponins display antitumorigenic effects by activating a variety of antitumor pathways[23]. The highest peak area, 19052.2 AU and the lowest peak area, 5158.5AU were observed at Rf of 0.03 and 0.66, respectively. Best solvent system to be observed for the above separation is chloroform: glacial acetic acid: methanol: water (6.4:3.2:1.2:0.8) (Tables 2 and 6, fig. 1d).

Terpenoids can be observed under daylight, 254 nm and 366 nm before derivatization and after derivatization, blue, bluish violet colour under visible light confirmed the presence of terpenoids in the sample and standard. Three different terpenoids were separated by visualising them in the Rf range of 0.57-0.76 (Tables 2 and 7 and fig. 1e). The highest and lowest peak areas, 3727.1 AU and 1782.0 AU were observed at the Rf of 0.64 and 0.57, respectively. Suitable solvent system determined

TABLE 2: VARIOUS SECONDARY METABOLITES OBSERVED IN HPTLC OF METHANOL EXTRACT OF THE STEM OF *SOLENA AMPLEXICAULIS*

Name of the compound	Mobile phase	Spray reagent	Colour of the spot/band	
			Visibile light	UV (366nm)
Alkaloids	Ethyl acetate-methanol-water (10:1.35:1)	Dragendorff's reagent followed by 10% ethanolic sulphuric acid reagent	Yellow, orange-yellow	Nil
Flavonoids	Toluene-acetone-formic acid (4.5 : 4.5 : 1)	1% Ethanolic aluminium chloride reagent	Nil	Yellow, yellowish blue
Glycosides	Ethyl acetate-ethanol-water (8 : 2 : 1.2)	Anisaldehyde sulphuric acid reagent	Pinkish violet	Nil
Saponins	Chloroform-glacial acetic acid-methanol-water (6.4 : 3.2 : 1.2 : 0.8)	Anisaldehyde sulphuric acid reagent	Blue, yellow, green, violet	Nil
Terpenoids	n-Hexane-ethyl acetate (7.2 : 2.9)	Anisaldehyde sulphuric acid reagent	Blue, bluish violet	Nil

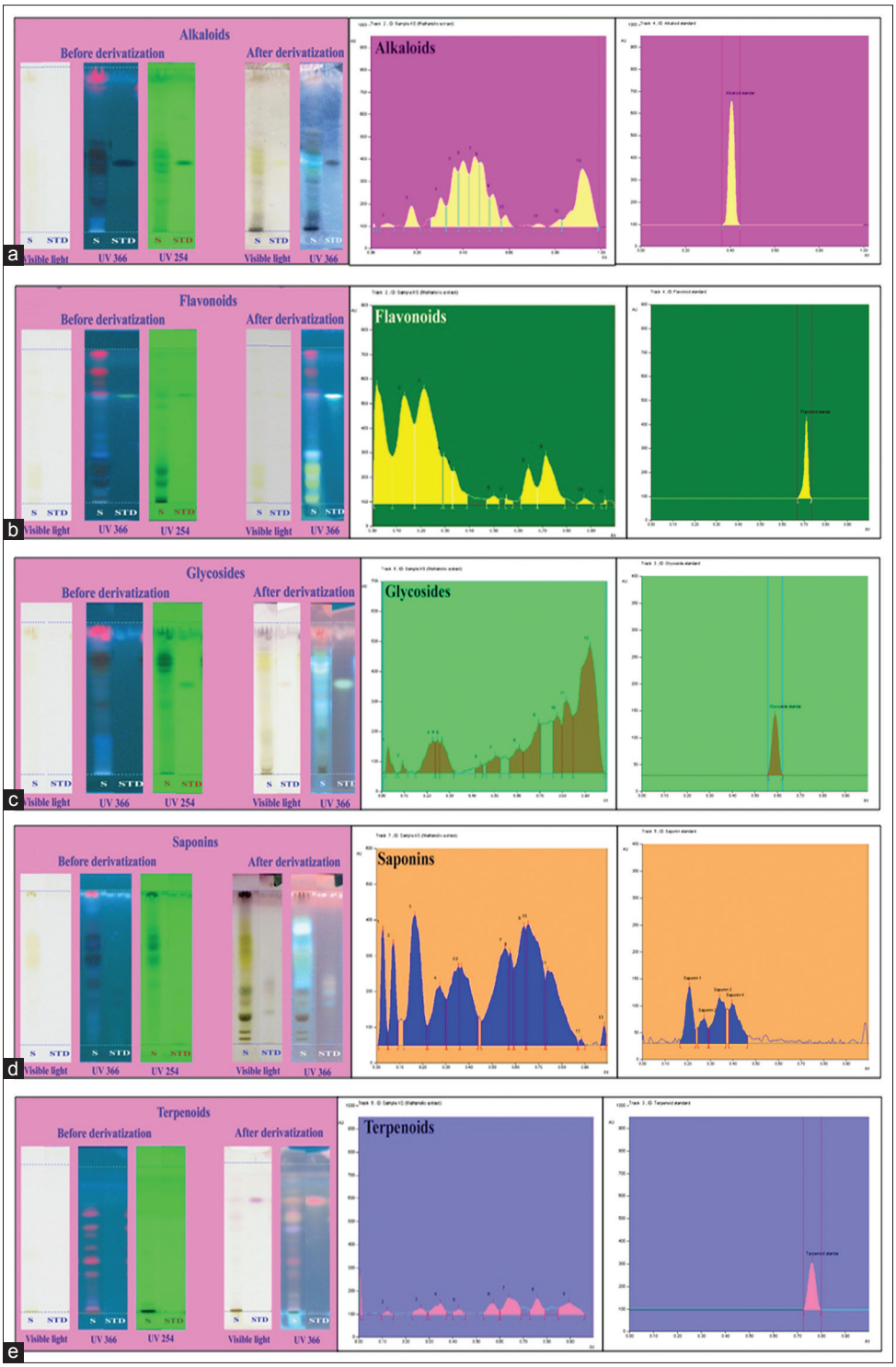

Fig. 1: HPTLC fingerprinting profile and densitogram of methanol extract of the stem of *Solena amplexicaulis*.
Fingerprinting profile and densitograms of different phytochemicals such as (a) alkaloids, (b) flavonoids, (c) glycosides, (d) saponins and (e) terpenoids separated from the methanol extract of the stems of *Solena amplexicaulis* using HPTLC.

was n-hexane: ethylacetate (7.2:2.9). More than 40 000 individual terpenoids are known to exist in nature with new compounds being discovered every year[24]. A large number of terpenoids exhibited cytotoxicity against a variety of tumor cell lines as well as anticancer efficacy in preclinical animal models[25,26]. Based on the results obtained in the study, we conclude that the methanol extract of stems of *S. amplexicaulis* has considerable amount of secondary metabolites, some of which could be

TABLE 3: ALKALOIDS IN THE HPTLC PROFILE OF THE METHANOL EXTRACT OF THE STEM OF *SOLENA AMPLEXICAULIS*

Peak	Rf	Height (mm)	Area (AU)	Assigned substance
1	0.01	15.5	82.2	Unknown
2	0.07	15.3	539.0	Unknown
3	0.18	95.0	2872.1	Alkaloid 1
4	0.30	131.3	5068.2	Alkaloid 2
5	0.36	267.4	9095.2	Alkaloid 3
6	0.40	295.2	11283.1	Alkaloid 4
7	0.45	315.2	10895.2	Unknown
8	0.48	289.5	8692.7	Alkaloid 5
9	0.53	146.8	4646.9	Unknown
10	0.59	52.2	1424.5	Alkaloid 6
11	0.73	12.7	431.4	Unknown
12	0.82	38.3	1000.6	Unknown
13	0.92	268.3	18796.9	Unknown
1	0.30	353.9	10774.5	Alkaloid standard

TABLE 4: FLAVONOIDS IN THE HPTLC PROFILE OF THE METHANOL EXTRACT OF THE STEM OF *SOLENA AMPLEXICAULIS*

Peak	Rf	Height (mm)	Area (AU)	Assigned substance
1	0.02	485.4	20704.0	Flavonoid 1
2	0.13	440.6	24890.9	Flavonoid 2
3	0.21	468.1	32643.4	Flavonoid 3
4	0.29	191.1	4995.5	Flavonoid 4
5	0.34	134.6	4114.9	Unknown
6	0.50	31.1	1045.7	Unknown
7	0.55	22.1	427.9	Flavonoid 5
8	0.64	144.1	5201.9	Flavonoid 6
9	0.71	199.0	9599.3	Unknown
10	0.87	21.7	639.7	Unknown
11	0.96	16.9	139.6	Unknown
1	0.71	364.8	7412.5	Flavonoid standard

TABLE 5: GLYCOSIDES IN THE HPTLC PROFILE OF THE METHANOL EXTRACT OF THE STEM OF *SOLENA AMPLEXICAULIS*

Peak	Rf	Height (mm)	Area (AU)	Assigned substance
1	0.03	84.7	1526.9	Unknown
2	0.09	32.2	484.5	Unknown
3	0.23	108.8	4099.3	Unknown
4	0.25	110.9	1697.0	Unknown
5	0.27	109.0	3185.4	Unknown
6	0.44	29.0	650.0	Unknown
7	0.50	58.7	2437.2	Unknown
8	0.61	83.3	3547.7	Unknown
9	0.69	166.5	7167.7	Glycoside 1
10	0.78	190.9	6110.6	Unknown
11	0.82	240.9	8708.6	Glycoside 2
12	0.92	423.5	29759.9	Unknown
1	0.59	122.0	3589.4	Glycoside standard

developed as pharmacotherapeutic agent in future. The fingerprints developed in this study are likely to aid in the quality control and standardization of herbal formulations containing this plant.

TABLE 6: SAPONINS IN THE HPTLC PROFILE OF THE METHANOL EXTRACT OF THE STEM OF *SOLENA AMPLEXICAULIS*

Peak	Rf	Height (mm)	Area (AU)	Assigned substance
1	0.03	323.3	5158.5	Saponin 1
2	0.07	284.5	5571.6	Saponin 2
3	0.17	363.8	16539.7	Saponin 3
4	0.27	165.4	8169.9	Saponin 4
5	0.35	219.1	8434.2	Saponin 5
6	0.37	219.1	10054.1	Saponin 6
7	0.56	270.5	16269.4	Saponin 7
8	0.58	259.4	4324.4	Unknown
9	0.64	332.0	11640.5	Saponin 8
10	0.66	337.7	19052.2	Saponin 9
11	0.74	207.9	12969.1	Unknown
12	0.89	16.3	219.9	Unknown
13	0.98	54.3	584.4	Unknown
1	0.21	105.7	327.3	Saponin standard 1
2	0.28	46.1	1464.1	Saponin standard 2
3	0.34	84.3	3715.2	Saponin standard 3
4	0.39	74.4	3075.9	Saponin standard 4

TABLE 7: TERPENOIDS IN THE HPTLC PROFILE OF THE METHANOL EXTRACT OF THE STEM OF *SOLENA AMPLEXICAULIS*

Peak	Rf	Height (mm)	Area (AU)	Assigned substance
1	0.01	181.6	652.1	Unknown
2	0.12	16.0	325.8	Unknown
3	0.27	27.8	969.1	Unknown
4	0.35	48.9	1913.8	Unknown
5	0.42	23.2	652.6	Unknown
6	0.57	50.2	1782.0	Terpenoid 1
7	0.64	74.0	3727.1	Terpenoid 2
8	0.76	69.6	2319.7	Terpenoid 3
9	0.89	51.5	3128.3	Unknown
1	0.71	143.0	4328.0	Terpenoid standard

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