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TLC and HPTLC Fingerprint Profiles of Different Bioactive Components from the Tuber of *Solena amplexicaulis*.

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ABSTRACT
Most of the traditional medicinal plants in India are not scientifically validated. Scientific evaluation along with traditional knowledge is essential to obtain effective drugs for commercial purpose. *Solena amplexicaulis* belongs to the family, Cucurbitaceae, a traditional medicinal plant species of Tamil Nadu, India is being prescribed to cure various diseases. The present study is to establish the chemical fingerprint through TLC and HPTLC analyses for various secondary metabolites in this species. The TLC and HPTLC 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 methanolic tuber extract showed the presence of 5 alkaloids, 6 flavonoids, 2 glycosides, 10 saponins and 7 terpenoids. The development of such fingerprint can be used in differentiation of the species from the adulterant in terms of phytochemical constituents and hence act as biochemical markers in the pharma industry and plant systematic studies.

Keywords: *Solena amplexicaulis*, tuber, TLC and HPTLC profiles, bioactive compounds.

1. Introduction
The current scenario exhibits the demand for plant drugs throughout the world because of its valuable phytochemicals. Phytochemical investigations are carried out during the 1970s and 1980s have discovered a number of alkaloids and other pharmacologically active substances that are currently being studied and that can possibly serve as models for new synthetic compounds [1]. Now a day's new technology have made it possible to identify, screen and isolate these active compounds. The chromatographic and spectral fingerprints play an important role in the quality control of complex herbal medicines [2]. Thin Layer Chromatography (TLC) is the first step to identify the phytochemical compounds present in the sample. The advancement of TLC, High Performance Thin Layer Chromatography (HPTLC) can provide an electronic image of chromatographic finger print and densitogram to detect the presence of a marker compound in the plant sample. Both the methods are more efficient, faster and the results are reliable and reproducible [3].
Solena amplexicaulis (Lam.) Gandhi is commonly called as creeping cucumber, belongs to the family, Cucurbitaceae. The medicinal uses of this species are multifaceted [4]. The traditional healers are prescribing the tubers of this species for various ailments like astringent, appetizer, carminative, cardiotonic, digestive, diuretic, expectorant, invigorating, purgative, stimulant, sour and thermogenic [5, 6]. The whole plant is a potential source of natural antioxidant [7, 8] antidiabetic agent [9] and antibacterial agent [10] also. As the leaves have the good anti-inflammatory property, the species is recognized as CNS active, diuretic, febrifuge and hypothermic [11, 6]. The 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 administered orally to cure stomachache [14]. The seeds are purgative [6].

With this background an attempt has been made to establish the phytochemical constituents like alkaloids, flavonoids, glycosides, terpenoids and saponins present in the methanolic tuber extract of *S. amplexicaulis* through TLC and HPTLC techniques. These studies helpful for the scientific verification of folklore claim with regard to the utility of this plant.

2. Materials and Methods
2.1 Collection, identification and preparation of plant material
The tubers of *S. amplexicaulis* were collected separately from the thorny scrub jungles of

Madukkarai, Coimbatore district, Tamil Nadu, India during March, 2013 (Figure 1). The authenticity of the plant was confirmed in Botanical Survey of India, Southern Regional Centre, Coimbatore by referring the deposited specimen (Voucher specimen number: CPS 313). They were washed thoroughly in tap water, shade dried and then homogenized to fine powder and stored in airtight bottles.

2.2 Preparation of extract

50 g of powdered tubers of *S. amplexicaulis* were extracted with 250 ml methanol at the temperature between 60 and 65°C for 24 h by using a soxhlet extractor. The solvent was evaporated by rotary vacuum evaporator to obtain viscous semi-solid masses. This semi-dry methanolic crude extract was subjected to TLC and HPTLC analysis.

2.3 Phytochemical analysis

TLC and HPTLC studies were carried out by following the methods of Harborne [15] and Wagner *et al.* [16] respectively.

2.4 TLC (Thin Layer Chromatography) profile

For the separation of different phytochemical compounds in the methanol extract of tuber of *S. amplexicaulis*, the extract was spotted manually using a capillary tube on precoated silicagel G TLC plates (15X5 cm with 3 mm thickness). The spotted plates were put into a solvent system to detect the suitable mobile phase as per the method of Wagner *et al.* [16, 17]. After the separation of phytochemical constituents, the spraying reagents such as Dragendorff reagent, 10% ethanolic sulphuric acid, 10% sulphuric acid, 5% ferric chloride, Kedde reagent, vanillin phosphoric acid reagent and vanillin sulphuric acid reagent were used to identify the respective compounds. The colour of the spots were noted and R_f values were calculated by using the following formula:

$$\text{Retention time (Rf)} = \frac{\text{Distance travelled by the solute}}{\text{Distance travelled by the solvent}}$$

2.5 HPTLC (High Performance Thin Layer Chromatography) profile

2.5.1 Sample preparation

100 mg of the tuber methanolic extract was dissolved in 1ml of HPTLC grade methanol and centrifuged at 3000 rpm for 5 min. This solution was used as a test solution for HPTLC analysis.

2.5.2 Developing solvent system

Different solvent systems were used to develop HPTLC fingerprint profile for different secondary metabolite groups *viz*; alkaloids, flavonoids, glycosides, terpenoids and saponins separately [15, 16, 18].

2.5.3 Sample application

2 µl of sample and 3 µl of standard solution were loaded as 5mm band length separately on precoated silica gel $60F_{254}$ aluminum sheets (3 x 10 cm) using a Hamilton syringe with the help of Linomat 5 applicator attached to CAMAG HPTLC system, which was programmed through WIN CATS software.

2.5.4 Development of chromatogram

After the application of spots, the chromatogram was developed in the twin trough glass chamber (20X10 cm) presaturated with respective mobile phase.

2.5.5 Detection of spot

The air dried plates were kept in a photo documentation chamber (CAMAG REPROSTAR 3) and captured the images in visible light, UV 366 nm and UV 254 nm. The chromatogram was scanned by the densitometer at 405 nm after spraying with respective spray reagent and dried at 100°C in hot air oven. The peak number with its height, area and R_f values of fingerprint data were recorded by WIN CATS (1.3.4 version) software.

Table 1: Determination of phytochemicals with suitable mobile phases through TLC in the methanolic tuber extract of the study species, *Solena amplexicaulis.*

Mobile phases	Spraying reagents	Colour of the spot/band	R_f	Compound
Chloroform-Methanol [3:2]	Dragendorff reagent / 10% ethanolic H_2So_4 reagent	Orange / Brown	0.19	Alkaloids
Ethylacetate-Methanol-Water-Glacial aceticacid [1.35:0.5:0.5:0.05] Ethylacetate-Methanol-Water-Toluene [1.4:0.5:0.5:0.05]	10% H_2So_4 / 5% ferric chloride solution	Yellow / Grey	0.78 0.85	Flavonoids
Ethylacetate-Methanol [1.3:0.5]	Kedde reagent	Distinct band formation	0.42	Glycosides
Chloroform-Methanol [1.2:0.2]	Vanillin H_2So_4 reagent	Violet blue	0.76	Saponins
Petroleumether-Ethylacetate [2:0.5] Hexane-Ethylacetate [1.5:0.5]	Vanillin phosphoric acid reagent	Blue	0.16 0.32	Terpenoids

Table 2: Mobile phase, spraying reagent used and the changed colour for various secondary metabolites by using HPTLC for methanolic tuber extract of *Solena amplexicaulis.*

Name of the compounds	Mobile phases	Spraying reagents	Colour of the spot/band	
			Visible 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

Table 3: HPTLC profile of the methanolic tuber extract of *Solena amplexicaulis* for alkaloids.

Peak	R_f	Height (mm)	Area (AU)	Assigned substance
1	0.01	16.2	117.0	Unknown
2	0.07	12.5	139.4	Unknown
3	0.14	155.0	9630.9	Alkaloid 1
4	0.29	211.2	9416.3	Alkaloid 2
5	0.37	74.3	2419.6	Unknown
6	0.44	311.0	14224.3	Alkaloid 3
7	0.52	417.7	18818.1	Alkaloid 4
8	0.57	106.1	2667.4	Alkaloid 5
9	0.71	54.9	2729.2	Unknown
10	0.81	14.3	327.8	Unknown
11	0.92	231.1	17715.8	Unknown
1	0.30	353.9	10774.5	Alkaloid standard

Table 4: HPTLC profile of the methanolic tuber extract of *Solena amplexicaulis* for flavonoids.

Peak	Rf	Height (mm)	Area (AU)	Assigned substance
1	0.02	245.1	7342.9	Flavonoid 1
2	0.25	309.5	26052.8	Flavonoid 2
3	0.33	150.4	3789.3	Flavonoid 3
4	0.43	64.9	2552.2	Flavonoid 4
5	0.50	14.5	429.8	Unknown
6	0.67	88.7	3064.3	Flavonoid 5
7	0.70	201.3	4012.4	Flavonoid 6
8	0.72	190.7	5075.6	Unknown
1	0.71	364.8	7412.5	Flavonoid standard

Table 5: HPTLC profile of the methanolic tuber extract of *Solena amplexicaulis* for glycosides.

Peak	R_f	Height (mm)	Area (AU)	Assigned substance
1	0.01	61.6	338.3	Unknown
2	0.03	10.5	57.6	Unknown
3	0.05	15.7	132.4	Unknown
4	0.14	113.0	5531.8	Unknown
5	0.19	75.7	1299.6	Unknown
6	0.22	84.8	1951.7	Unknown
7	0.28	61.3	1598.7	Unknown
8	0.42	48.7	1311.8	Unknown
9	0.43	48.1	901.5	Unknown
10	0.50	58.9	1803.8	Unknown
11	0.58	88.6	4774.3	Unknown
12	0.65	74.9	2338.2	Unknown
13	0.74	292.7	13755.2	Glycoside 1
14	0.79	114.8	1844.6	Unknown
15	0.82	206.3	7369.8	Glycoside 2
16	0.90	300.6	18147.4	Unknown
1	0.59	122.0	3589.4	Glycoside standard

Table 6: HPTLC profile of the methanolic tuber extract of *Solena amplexicaulis* for saponins.

Peak	R_f	Height(mm)	Area(AU)	Assigned substance
1	0.03	118.2	1797.0	Saponin 1
2	0.07	257.9	5842.4	Saponin 2
3	0.12	202.6	5297.0	Saponin 3
4	0.15	275.1	5891.5	Unknown
5	0.17	278.9	11057.3	Saponin 4
6	0.26	247.5	6928.3	Saponin 5
7	0.36	389.0	36132.4	Saponin 6
8	0.50	184.0	5074.3	Saponin 7
9	0.56	254.9	9679.8	Saponin 8
10	0.65	426.6	31002.3	Saponin 9
11	0.74	212.5	11370.6	Saponin 10
12	0.88	33.6	468.6	Unknown
13	0.98	97.4	1305.7	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

3. Results

The present study was oriented towards the phytochemical screening of the species, *S. amplexicaulis* and development of fingerprints using TLC and HPTLC technique.

3.1 TLC (Thin Layer Chromatography) profile

The methanolic tuber extract was subjected to TLC, different compositions of the mobile phase were tried in order to separate the different secondary metabolites like alkaloids, flavonoids, glycosides, terpenoids and saponins (Table 1, Figure 2). The samples were spotted on the TLC plates which were developed in the appropriate solvent system. After derivatization with proper spraying reagent, the colour developed was noted. Based on the colour, the secondary metabolites were differentiated and R_f values were calculated. The study revealed that relatively high polarity solvents *viz.*, chloroform, ethyl acetate and methanol were most fit as mobile phases for the separation of bioactive compounds in the tubers of *S. amplexicaulis.*

3.2 HPTLC (High Performance Thin Layer Chromatography) profile

HPTLC profile of methanolic extract 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).

Table 7: HPTLC profile of the methanolic tuber extract of *Solena amplexicaulis* for terpenoids.

Peak	R_f	Height (mm)	Area (AU)	Assigned substance
1	0.01	199.2	1086.1	Unknown
2	0.18	12.4	75.1	Unknown
3	0.24	15.3	234.4	Unknown
4	0.27	25.7	453.9	Unknown
5	0.31	25.4	697.7	Unknown
6	0.56	51.6	1907.8	Terpenoid1
7	0.65	34.7	1021.9	Terpenoid 2
8	0.67	36.3	829.5	Unknown
9	0.78	42.4	2043.6	Terpenoid 3
10	0.87	51.4	1905.9	Unknown
11	0.93	62.8	1676.1	Terpenoid 4
1	0.71	143.0	4328.0	Terpenoid standard

Fig 1: Tubers of *Solena amplexicaulis* (Lam.) Gandhi. (Cucurbitaceae)

3.2.1 Alkaloids
The HPTLC chromatogram can be best observed under daylight, UV254 nm and 366 nm before and after derivatization. 5 bands of different types of alkaloids were seen before derivatization at visible mode. The highest peak area is 18818.1AU and the lowest peak area, 2667.4AU were observed at R_f of 0.52 and 0.57 respectively. Best solvent system to be observed for the above separation is Ethyl acetate: Methanol: Water (10:1.35:1) (Tables 2 and 3, Figure 3a).

3.2.2 Flavonoids
HPTLC profile of the methanolic tuber extract of *S. amplexicaulis* was recorded in Tables 2 and 4, Figure 3b. Yellow or yellowish blue coloured fluorescence zone at UV366 nm mode was observed from the chromatogram after derivatization, which confirmed the presence of flavonoids in the sample and standard. 8 compounds were separated and among them 6 were flavonoids at the R_f in the range of 0.02 to 0.70. The highest peak area was 26052.8AU and that of the lower was 2552.2AU observed at R_f of 0.25 and 0.43 respectively.

3.2.3 Glycosides
After derivatization, the pinkish violet colour confirmed the presence of glycosides in the given samples and standard. The methanolic tuber extract revealed totally 16 spots and among them only 2 bands were glycosides in the R_f level of 0.74 and 0.82 (Tables 2 and 5, Figure 3c). A most appropriate solvent system for the above separation was Ethyl acetate: Ethanol: Water (8:2:1.2).

3.2.4 Saponins
Saponins can be observed at daylight, 254 nm and 366 nm before derivatization. After derivatization blue, yellow, green and violet colours at visible light confirmed the presence of saponins in the sample and standards. 10 different saponins were separated by seeing in the R_f range of 0.03 to 0.74 (Tables 2 and 6, Figure 3d). The highest and lowest peak areas 36132.4AU and 1797.0AU were observed at the R_f of 0.36 and 0.03 respectively. The suitable solvent system determined to be Chloroform: Glacial acetic acid: Methanol: Water

(6.4:3.2:1.2:0.8).

3.2.5 Terpenoids: 4 different types of terpenoids were observed out of 11 bands in the methanolic tuber extract of *S. amplexicaulis*. The R_f values determined for the terpenoids were in the range of 0.56 – 0.93 (Tables 2 and 7, Figure 3e). The best solvent system evaluated was n-Hexane: Ethyl acetate (7.2:2.9). Blue and bluish violet colour confirmed the presence of terpenoids in the sample and standard. The highest peak area was 2043.6AU and that of the lowest one was 1021.9AU which were observed at R_f of 0.56 and 0.93.

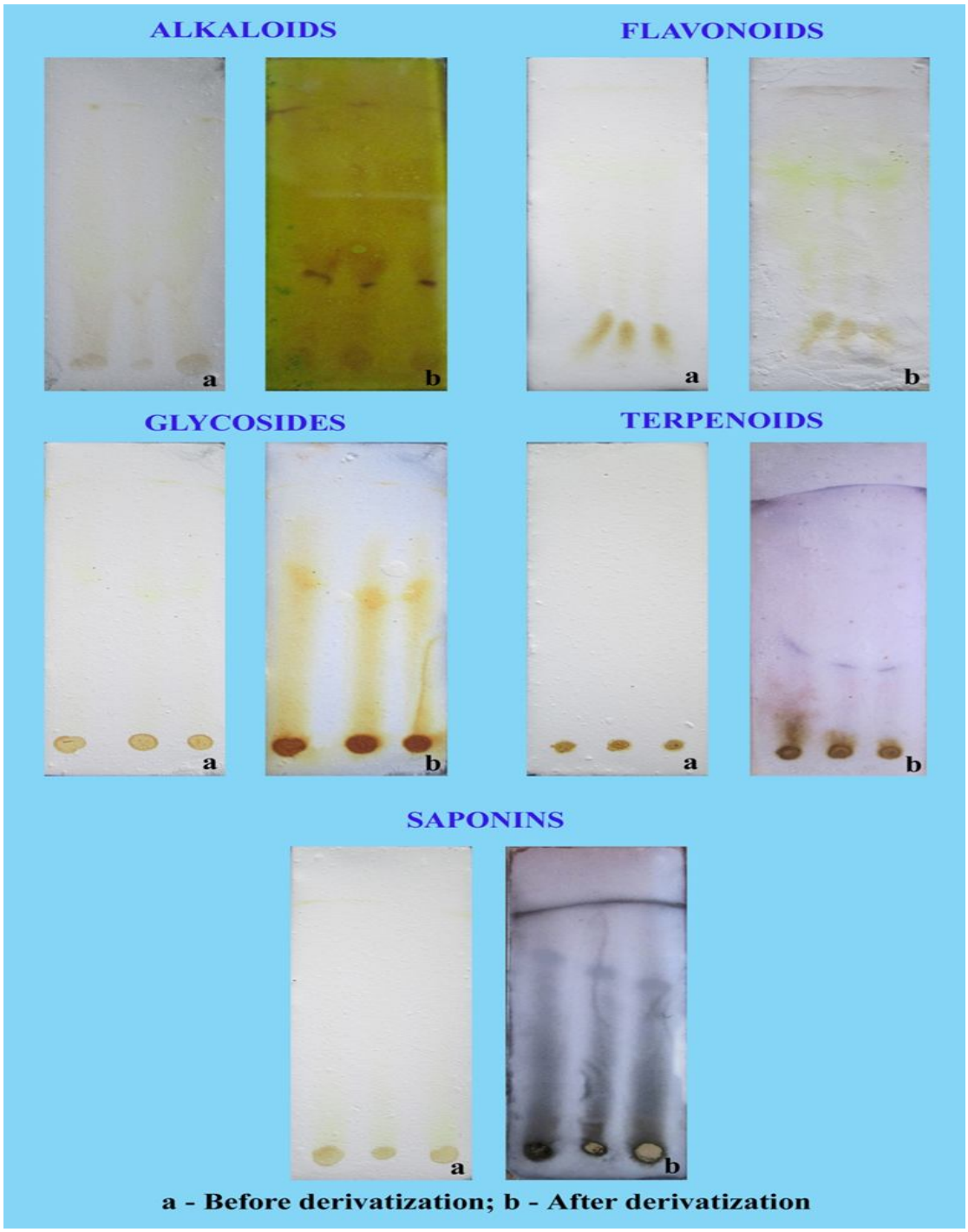

Fig 2: TLC fingerprinting profile of methanolic tuber extract of *Solena amplexicaulis* for different phytochemicals.

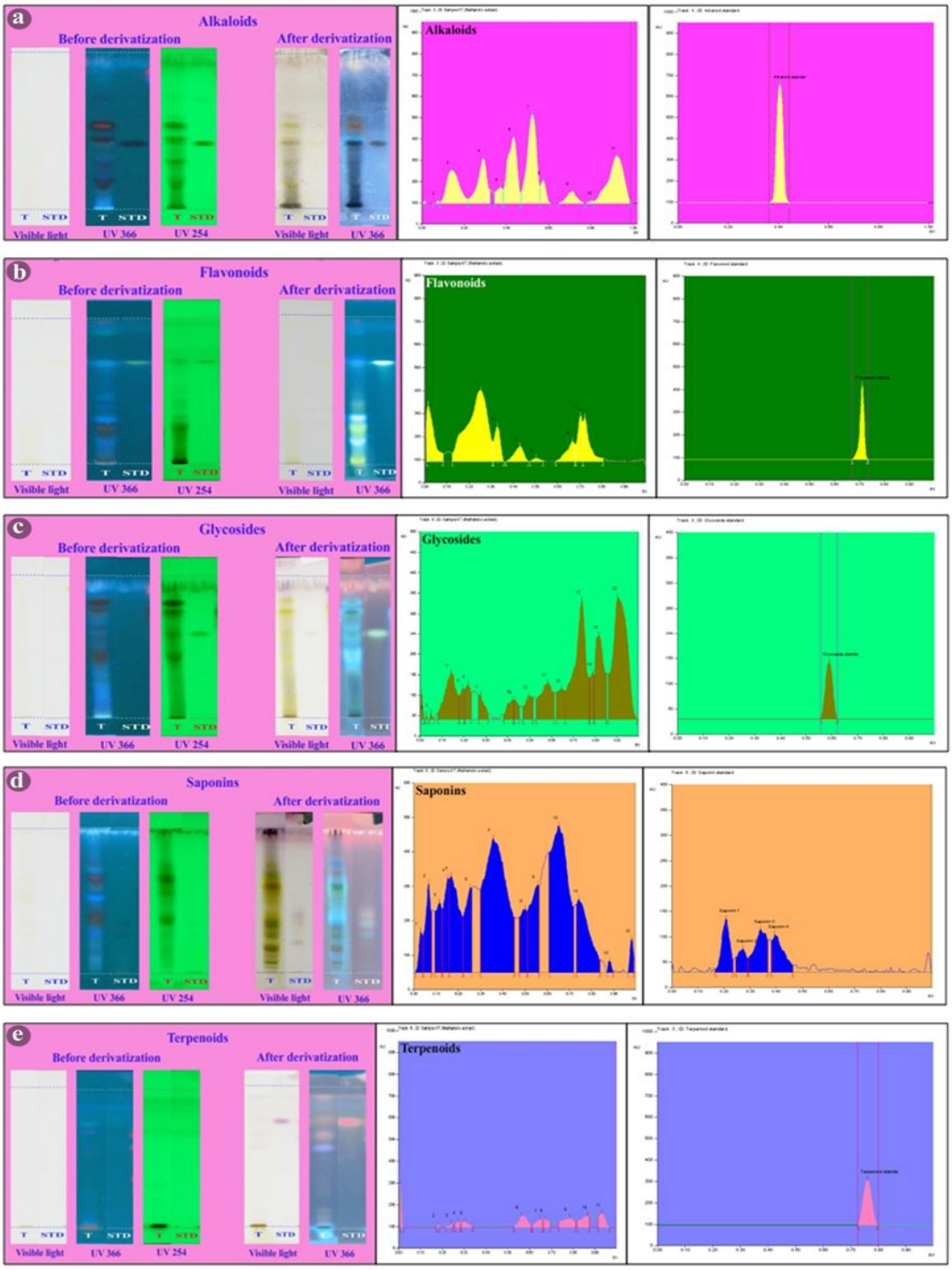

Fig 3(a-e): HPTLC fingerprinting profile and densitogram of methanolic tuber extract of *Solena amplexicaulis* and respective standard for different phytochemicals. Figures in column 2 and 3 are the densitogram of samples and standards respectively.

4. Discussion

The medicinal plants are rich in secondary metabolites and among the vast array of bioactive compounds alkaloids, flavonoids, glycosides, saponins and terpenoids are in high interest. Of the several methods are available for separating plant constituents, the chromatographic procedure is the most commonly used techniques for general application [19]. The present TLC and HPTLC studies confirmed the presence of active metabolites in the methanolic tuber extract of the study species, *S. amplexicaulis.*

In TLC, the methanolic tuber extract of *S. amplexicaulis* showed the presence of alkaloids, flavonoids, glycosides,

saponins and terpenoids in different solvent compositions with different R_f levels (Table 1). The HPTLC also confirmed the presence of 5 alkaloids, 6 flavonoids, 2 glycosides, 10 saponins and 4 terpenoids with different R_f values and peak areas (Table 3-7). The mobile phases used for TLC and HPTLC were relatively high polar solvents *viz*; chloroform, ethyl acetate and methanol to separate the bioactive compounds (Table 1 and 2). Many early reports are also suggesting this mobile phase of high polarity solvents for effective separation of the bioactive compounds in many plant species [20, 21, 22].

Alkaloids are one of the most abundant and diverse group of secondary metabolites found at 10-15% concentration in almost all plants. Several of them have been known to mankind for several thousand years. Alkaloids disrupt the integrity of bio membrane, potent inhibitors of ion channel and impair the function of microtubules or microfilaments. It also possesses antimitotic and allergic effects at the cellular level. Many alkaloids, though poisonous, have a physiological effect that renders them valuable medicine against various diseases, including malaria, diabetes, cancer, cardiac dysfunction *etc*. These are also used in local anesthesia and relief of pain [23]. Common medicines derived from alkaloids include, atropine derived from the nightshade *Atropa belladonna*, quinine from the Cinchona tree *etc* [24]. In this present investigation, 5 different types of alkaloids are identified with different Rf levels.

Flavonoids are plant pigments that are synthesized from phenylalanine, generally display marvelous colors known from flower petals, mostly emit brilliant fluorescence when they are excited by UV light, and are ubiquitous to green plant cells. It inhibits many bacterial strains, inhibit important viral enzymes, such as reverse transcriptase and protease, and destroy some pathogenic protozoans. It has ability to inhibit specific enzymes, to stimulate some hormones and act as a neurotransmitters [25]. Most flavonoids function in the human body as antioxidants [26] and control inflammation [27]. Common examples for flavonoids are quercetin, rutin, hesperidin *etc*. The methanolic tuber extract of S. *amplexicaulis* revealed 6 different types of flavonoids with different Rf levels.

Glycosides play numerous important roles in living organisms. It occurs in plants, often as flower and fruit pigments for example, anthocyanins. Flavonoid glycosides are yellow pigments in flowers and plants which have demonstrated anti-inflammatory, anti-allergic effects, antithrombotic and antidiarrheal properties [28]. Cyanogenic glycosides, which initially contain hydrogen cyanide (HCN) compounds, are toxic to unadapted farm animals and humans [29]. Cardiac glycosides do exert a specific action on the myocardial muscle and allay myocardial infarction. Coumarin glycosides have been shown to have hemorrhagic, ant fungicidal, and antitumor activities. Lactone glycoside dicumarol is known as an anticoagulant [30]. From the results among the 16 bands detected only 2 were observed as glycosides.

Saponins are glycosides of triterpenes, steroids or steroidal alkaloids. They can be found in plants and marine organisms. Very diverse biological activities are ascribed to saponins and they play important roles in food, animal feedstuffs, and pharmaceutical properties [31]. Saponins traditionally used as a natural detergent. They also exhibit a variety of biological activities like anti-inflammatory [32], hypocholesterolemic [33] and immune-stimulating [34] whose properties are widely recognized and commercially utilized. The research on saponins in various forms as a treatment for cancer has generated a lot of potential [35]. In the present study, good separation of saponins has also been observed in the species, *S. amplexicaulis*. Totally 10 different types of saponins were separated.

Terpenoids are also called as isoprenoids mainly occur in plants. More than 40,000 individual terpenoids are known to exist in nature with new compounds being discovered every year. Most of the terpenoids are of plant origin; however, they are also synthesized by other organisms, such as bacteria and yeast as part of primary or secondary metabolism. Terpenoids have been found to be useful in the prevention and therapy of several diseases, including cancer, antimicrobial, antiparasitic, antiviral, anti-allergenic, antispasmodic, antihyperglycemic, anti-inflammatory, and immunomodulatory properties [36, 37]. A large number of terpenoids exhibit cytotoxicity against a variety of tumor cells and cancer preventive as well as anticancer efficacy in preclinical animal models [38]. Some important terpenes include camphor, menthol *etc*. The tuber extract showed four different kinds of terpenoids with the Rf ranging of 0.56 to 0.93.

The developed TLC and HPTLC methods will help the manufacturer for quality control and standardization of herbal formulations, such finger printing is useful in differentiating the species from the adulterant and act as biochemical markers for this medicinally important plant species in the Pharma industries and plant systematic studies [39]. This report justifies the medicinal usage of this species by the rural people of the western districts of Tamil Nadu.

5. Conclusion

This report confirmed the presence of the rich variety of bioactive compounds in the species, *S. amplexicaulis* and it leads for the development of the new pharmaceuticals that address hither to unmet therapeutic needs. For further study, with the help of developing analytical method pure active chemical compound should be isolated and identified on the basis of reference standards.

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