Kong. Res. J. 1(1) : 117-121, 2014
Kongunadu Arts and Science College, Coimbatore

DOI:10.26524/krj25

PHYTOCHEMICAL STUDIES ON THE TERPENOIDS OF MEDICINALLY IMPORTANT PLANT *SOLANUM VILLOSUM* (MILL.) USING HPTLC

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ABSTRACT

To determine terpenoid profile of *Solanum villosum* using high performance thin layer chromatography (HPTLC) technique. 2cl of test solution and 2cl of standard solution was loaded as 6mm band length in the 3 x 10 Silica gel 60F254 TLC plate using Hamilton syringe and CAMAG LINOMAT 5 instrument. The extract was run along with the standard terpenoid compound and it was observed that the extract showed the presence of terpenoid and it was confirmed from the chromatogram after derivatization. The Rf value of the different compounds present in the extract was found to 0.04,0.21,0.27,0.55,0.59,0.65,0.75,0.80,0.90 and 0.94 of peak 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10 respectively. Among them, peaks 2, 6, 7 and 10 were found to be terpenoid compounds. It can be concluded that HPTLC analysis of ethanolic leaf extract of *Solanum villosum* (Mill) can be used as a diagnostic tool for the correct identification of the plant and it is useful as a phytochemical marker and also a good estimator of genetic variability in plant populations.

Key words: HPTLC, ethanol extract, *Solanum villosum*, phytochemicals, terpenoid.

1. INTRODUCTION

Solanum villosum (Mill.) belongs to family Solanaceae, it is commonly known as red-fruit nightshade, is widely distributed in many parts of India. *Solanum* is one of the most important and largest genera of the family Solanaceae comprising of about 84 genera and 3000 species were identified throughout the worldwide. The plant is an ayurvedic herb with multiple medicinal properties (Nandita Chowdhury *et al*., 2008).

The plant *Solanum villosum* contain many primary and secondary metabolites such as, alkaloids, flavonoids, phenols, saponins, tannins, terpenoids, steroids, carbohydrates, glycosides, amino acids and proteins. The genus of *Solanum* species contains excellent antioxidant properties and free radical scavenging ability (Annie Jacob and Radha, 2013).

The plants of *S. nigrum* complex has been traditionally used as an analgesic, antispasmodic, antiseptic, antidysentric, antinarcotic, emollient, diuretic, tonic, soporific, laxative, anticancer, antiulcer and for disorders of neuro-vegetative system etc. (Edmonds and Chweya, 1997). This medicinal value is mainly attributed to the alkaloid content of the plants.

The plant compounds mainly used for treating worms, cold, hoarseness of voice, fever, dysuria, enlargement of the liver, muscular pain, spleen and stone in the urinary bladder (Akilesh Sharma *et al*., 2011; Watt and Breyer-Brandwijk 1962; Aslanov and Novruzov 1978).

In the present study, HPTLC analysis of ethanolic leaf extract of *Solanum villosum* specifically for terpenoid profile was compared with the standards. The *Solanum villosum* and related species are widely used as leafy herbs and vegetables, as a source of fruit and for various medicinal purposes. In spite of known uses in traditional medicines, no documented evidence is available on terpenoid compound analysis. So the HPTLC analysis is to provide information for terpenoid content of the plant extract.

2. MATERIALS AND METHODS

2.1. Plant material

The leaves of the *Solanum villosum* (Mill.) plant were collected from Thadagam hills at Coimbatore district, Tamilnadu, India. The specimen sample was authenticated by Dr.V.S.Ramachandran, Associate Professor, Department of Botany, Bharathiar University, Coimbatore, Tamilnadu, India. The voucher specimen was deposited in the herbarium center, Department of Botany, Bharathiar University, Coimbatore.

2.2. Extraction of plant material

Plant materials thoroughly washed and shade dried at room temperature after that grind into powder was packed with No.1 Whatman filter paper and placed in soxhlet apparatus along with ethanol. The crude extract were collected and dried at room temperature, 30˚C after which yield was weighed and then performed.

2.3. HPTLC analysis of ethanolic extract of Solanum villosum (Mill)

Test solution preparation: The given ethanol extract 100mg was weighed in an electronic balance (Afcoset) and dissolved in 1ml ethanol and centrifuged at 3000rpm for 5min. This solution was used as test solution for HPTLC analysis.

2.4. Sample application

2cl of test solution and 2cl of standard solution was loaded as 6mm band length in the 3 x 10 Silica gel 60F254 TLC plate using Hamilton syringe and CAMAG LINOMAT 5 instrument.

2.5. Spot development

The samples loaded plate was kept in TLC twin trough developing chamber (after saturated with Solvent vapor) with respective mobile phase (Terpenoid) and the plate was developed in the respective mobile phase up to 90mm.

2.6. Photo-documentation

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 White light, UV 254 nm and UV366 nm.

2.7. Derivatization

The developed plate was sprayed with respective spray reagent (Terpenoid) and dried at 100°C in Hot air oven. The plate was photo-documented in Day light and UV 366nm mode using Photo-documentation (CAMAG REPROSTAR 3) chamber. Scanning Before derivatization, the plate was fixed in scanner stage (CAMAG TLC SCANNER 3) and scanning was done at UV 254nm. The Peak table, Peak display and Peak densitogram were noted. The software used was winCATS 1.3.4 version.

2.8. Analysis details for Terpenoid

Mobile phase: n-Hexane - Ethyl acetate (7.2 : 2.9). Spray reagent: Anisaldehyde sulphuric acid reagent.

3. RESULTS AND DISCUSSION

3.1. HPTLC analysis of Terpenoids

3.1.1. Detection

Blue, bluish violet coloured zones at Visible light mode present in the given standard and sample track observed in the chromatogram after derivatization, which confirmed the Presence of Terpenoid in the given standard and maybe in sample.

The ethanolic leaf extract of *Solanum villosum* was run along with the standard terpenoid compound and it was observed that the extract showed the presence of terpenoid and it was confirmed from the chromatogram after derivatization. The Rf value of the different compounds present in the extract was found to 0.04, 0.21, 0.27, 0.55, 0.59, 0.65, 0.75, 0.80, 0.90 and 0.94 of peak 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10 respectively. Among them, peaks 2, 6, 7 and 10 were found to be terpenoid compounds.

The WHO has emphasized the need to ensure the quality of medicinal plant products by using modern controlled techniques and applying suitable standards (WHO, 1998). Modern chromatographic techniques like HPLC and HPTLC were used to judge the authenticity of traditional recommendations (Khan *et al*., 2009). The HPTLC method can be used for phytochemical profiling of plants and quantification of compounds present in plants, with increasing demand for herbal products as medicines and cosmetics, there is an urgent need for standardization of plant products (Pawar *et al*., 2010).

HPTLC finger print analysis has become the most potent tool for quality control of herbal medicines because of its simplicity and reliability. It can serve as a tool for identification, authentication and quality control of herbal drug (Mauji *et al*., 2011). HPTLC finger printing profile is useful as phytochemical marker and also a good estimation of genetic variability in plant populations. Thus the HPTLC fingerprint profiles of the major chemical constituents in the crude extract along with their Rf values and percentage proportions were recorded which would serve as a reference standard for the scientist who engaged in research on the medicinal properties of this plant (Johnson *et al*., 2011).

The phytochemical evaluation is one of the tools for the quality assessment, which includes preliminary phytochemical screening, chemo profiling and marker compound analysis using modern analytical techniques. In the last two decades, HPTLC has emerged as an important tool for the qualitative, semiqualitative and quantitative phytochemical analysis of herbal drugs and formulations. The major advantage of HPTLC is that several samples can be analysed simultaneously using a small quantity of mobile phase (Modi *et al*., 2008). HPTLC fingerprint profiles of the *Solanum villosum* leaf extract for terpenoids have been developed. Rf values and the relative percentage of the separated compounds were recorded.

Table 1. HPTLC analysis of ethanolic leaf extract of *Solanum villosum* for Terpenoid profile

Track	Peak	Rf	Height	Area	Assigned substance
STD	1	0.85	90.8	3196.5	Terpenoid standard
Sample A	1	0.04	541.7	13312.1	Unknown
Sample A	2	0.21	27.1	601.7	Terpenoid 1
Sample A	3	0.27	85.7	2998.0	Unknown
Sample A	4	0.55	11.4	112.9	Unknown
Sample A	5	0.59	31.1	1464.8	Unknown
Sample A	6	0.65	15.3	270.2	Terpenoid 2
Sample A	7	0.75	66.0	1939.4	Terpenoid 3
Sample A	8	0.80	65.9	2166.5	Unknown
Sample A	9	0.90	26.9	868.9	Unknown
Sample A	10	0.94	21.4	459.8	Terpenoid 4

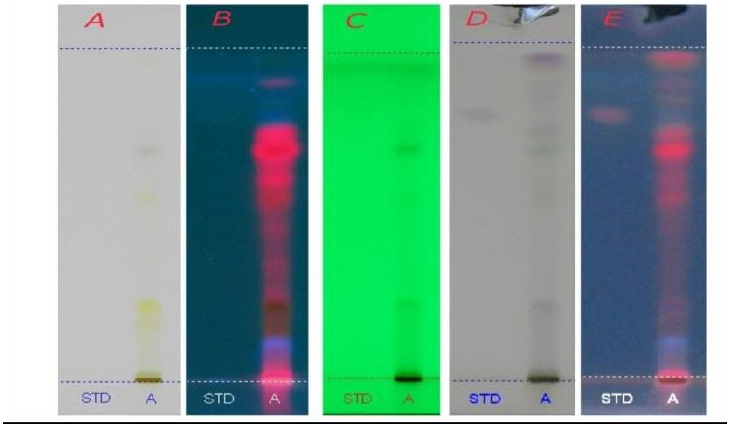

Figure 1. HPTLC studies on the terpenoid of medicinally important plant *S. villosum*

A: HPTLC of the ethanolic leaf extract of *S. villosum* under daylight. **B:** HPTLC of the ethanolic leaf extract of *S. villosum* under UV 366 nm **C:** HPTLC of the ethanolic leaf extract of S. villosum under UV 254 nm. **D:** HPTLC of the ethanolic leaf extract of *S. villosum* under daylight – after derivatization. **E:** HPTLC of the ethanolic leaf extract of *S. villosum* under UV 366 nm after derivatization.

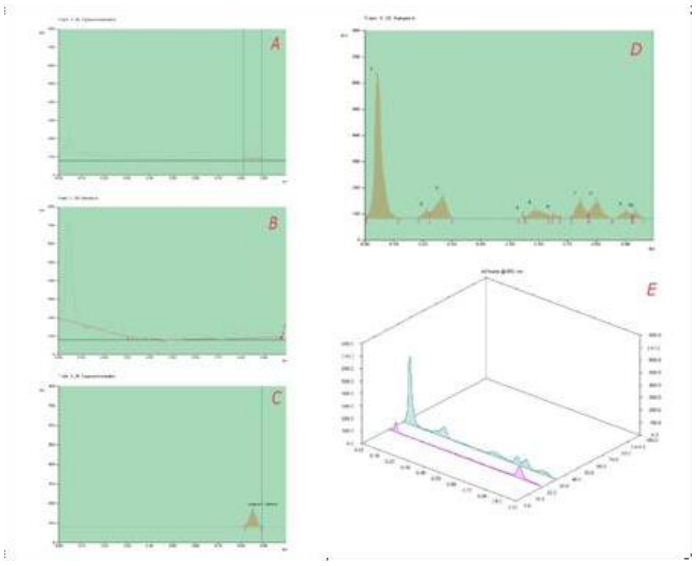

Figure 2. HPTLC chromatogram of ethanolic leaf extract of *S. villosum* (Mill.)

A: HPTLC chromatogram of Track STD – Terpenoid standard Baseline display (Scanned at 366nm), **B:** HPTLC chromatogarm of Track STD – Terpenoid standard Peak densitogram display (Scanned at 366nm), **C:** HPTLC chromatogarm of Track A – Sample A ethanolic leaf extract of *S.villosum* - Baseline display (Scanned at 366nm), **D:** HPTLC chromatogram of Track A – Sample A ethanolic leaf extract *S. villosum* - Peak densitogram display (Scanned at 500 nm), **E:** HPTLC chromatogram of 3D display of all Tracks.

Terpenoids composed of "isoprenoid" units constitute one of the largest group of natural products accounting for more than 40 000 individual compounds, with several new compounds being discovered every year (Sacchettini and Poulter, 1997; Peñuelas and Munné-Bosch, 2005; Withers and Keasling, 2007). 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 are synthesized from two five-carbon building blocks, i.e., the isoprenoid units. Based on the number of building blocks, terpenoids are classified into several

classes, such as monoterpenes (e.g., carvone, geraniol, *d*-limonene, and perillyl alcohol), diterpenes (e.g. retinol and *trans*-retinoic acid), triterpenes e.g., betulinic acid (BA), lupeol, oleanic acid, and ursolic acid (UA), and tetraterpenes e.g., α-carotene, β-carotene, lutein, and lycopene (Rabi and Bishayee, 2009).

The diverse array of terpenoid structures and functions has provoked increased interest in their commercial use. Terpenoids have been found to be useful in the prevention and therapy of several diseases, including cancer, and also to have antimicrobial, antifungal, antiparasitic, antiviral, anti-allergenic, antispasmodic, antihyperglycemic, antiinflammatory, and immunomodulatory properties (Wagner and Elmadfa, 2003; Sultana and Ata, 2008; Shah *et al*., 2008). In addition, terpenoids can be used as protective substances in storing agriculture products as they are known to have insecticidal properties (Theis and Lerdau, 2003).

4. CONCLUSION

It can be concluded that HPTLC analysis of ethanolic leaf extract of *Solanum villosum* (Mill.) for terpenoid profile can be used as a diagnostic tool for the correct identification of the plant and it is useful as a phytochemical marker and also a good estimator of genetic variability in plant populations.

5. ACKNOWLEDGEMENTS

The authors are thankful to our Secretary and Joint Secretary of Kongunadu Arts and Science College, Coimbatore, Tamilnadu, India for providing facilities and encouragement.

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