Thin layer chromatography (TLC) analysis of *Psoralea corylifolia* leaf extract

S RANJITH KUMAR[1], K CHOZHAN[2], R RAMAMOORTHY[1] and G SWATHIGA[1]

[1]Department of Sericulture, Forest College and Research Institute
Tamil Nadu Agricultural University, Mettupalayam 641301 Tamil Nadu, India
[2]Department of Crop Protection, Agricultural College and Research Institute
Tamil Nadu Agricultural University, Eachangkottai, Thanjavur 614902 Tamil Nadu, India
Email for correspondence: ranjithseri2012@gmail.com

© Society for Advancement of Human and Nature (SADHNA) ***Received: 09.07.2022/Accepted: 02.08.2022***

ABSTRACT

Apart from the fact that key constituents of *Psoralea corylifolia* performed a wide spectrum of biological functions, the precise biological actions of the plant chemical constituents have remained elusive and just a few chemicals identified from this plant have been used for medication. With the medicinal properties of *P corylifolia* in mind, the present study was aimed to identify the fractions of the compounds of the leaves of this plant. A fast and simple procedure for the determination of the main active principle of medicinal plant is proposed. The extraction of active compounds was performed by means of three solvents based on a method of increasing polarity. The plant extract with known compositions was analysed on silica gel layers with the aid of three solvent systems. Different R*f* values of the compound also reflected an idea about their polarity. The study will help in selection of appropriate solvent system for further separation of compounds from the plant extract.

Keywords: *Psoralea corylifolia*; R*f* values; solvent; plant extract; TLC

INTRODUCTION

Psoralea corylifolia, often known as Babchi, is a prominent herb that has been utilized in traditional Ayurvedic and Chinese medicine for its miraculous benefits in the treatment of numerous skin disorders for a long time. Chemoprotective, antioxidant, antibacterial and anti-inflammatory effects of this plant are also being researched pharmacologically (Zhang et al 2016). Apart from the fact that key constituents of *P corylifolia* performed a wide spectrum of biological functions, the precise biological actions of the plant chemical constituents have remained elusive and just a few chemicals identified from this plant have been used for medication. Thin layer chromatographic studies are necessary to separate the nature of active principles from the plant extracts (Sadasivam and Manickam 2005).

Extraction of plant molecules with previously optimized high and low polarity solvent mixtures, isolation of crude extract using analytical thin layer chromatography (TLC), biological screening of the extract for antioxidant and antimicrobial activities using TLC bioautography and purification of active compounds by preparative TLC are the approaches of thin layer chromatography (Rajauria and Abu-Ghannam 2013).

MATERIAL and METHODS

Thin layer chromatographic studies were conducted to separate the active compounds from *P corylifolia*.

Preparation of plant extract: Twenty five g of the powdered leaves of medicinal plant *P corylifolia* were weighed separately in chloroform and percolated overnight. The sample tube of the unit was fitted with a filter disc at the bottom and filled with ground samples, sealed with another filter disc and compressed. This was fitted to an electric heating mantle with Soxhlet unit, filled with 250 ml chloroform and a temperature of 60ºC was maintained for 6 h. The residual extract was collected in a flask and transferred to a rotary flask vacuum evaporator for evaporation of the solvent.

The residue thus obtained was stored at 4°C in airtight bottles for future use.

Preparation and activation of TLC plates: Silica gel-G (E-Merck) was used for preparing TLC plate of dimension 20 cm x 20 cm. Twenty five g of finely powdered silica gel was mixed thoroughly with 40 ml of distilled water. The slurry was poured into TLC applicator which was adjusted for 0.5 mm thick wet silica gel. The glass plate was allowed to dry in open air for 1 h and then heated in hot air oven at 110ºC for 2 h. The activated plate was loaded with 20 µl of the sample using a micropipette without disturbing the silica gel layer (Urzua et al 2008).

Separation of compounds through TLC: Using the capillary tube, 20 µl of the sample was applied on the activated plates and run separately for 20 min in the solvent system chloroform:methanol (09:01), chloroform:acetone (7.5:2.5) and chloroform alone (10:0). Compounds were detected by spraying with 1 per cent ferric chloride, idoplatinate and ninhydrin solution. Presence of compound was indicated by specific colour spots. The TLC plate was sprayed with the revealing agent in order to identify the compound present in the leaf extract. The TLC plate was dried and observed for the presence of colored spots which indicated the presence of phenolics, amino acids and polysaccharides (Manilal et al 2010, Vijayabaskar and Shiyamala 2011). All the spots were observed under UV light (254 nm). The relation to front (R*f*) of the spots developed on the TLC plate was recorded using the formula given below:

$$R_f \text{ value} = \frac{\text{Distance moved by the solute from the origin}}{\text{Distance moved by the solvent from the origin}}$$

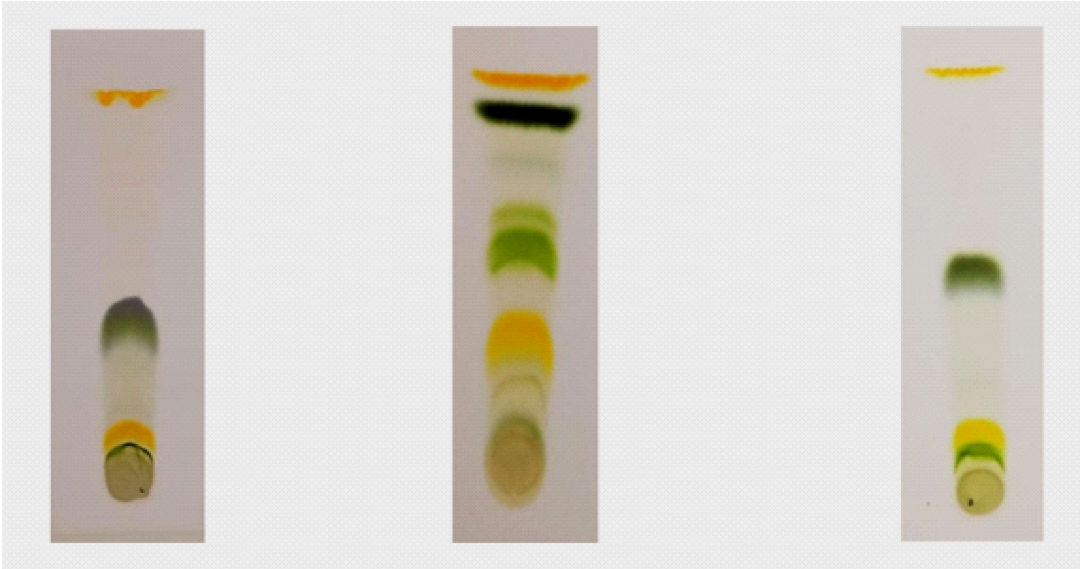
RESULTS and DISCUSSION

Thin layer chromatography (TLC) is an important method for separation, identification and characterization of various classes of plant bioactive compounds (Swaroop et al 2016). The identification of antimicrobial compounds of *P corylifolia* by TLC studies revealed that the mobile phase chloroform:methanol (09:01) eluted maximum of 17 distinct spots and their R*f* values were 0.04, 0.12, 0.16, 0.23, 0.25, 0.30, 0.33, 0.44, 0.50, 0.54, 0.61, 0.66, 0.70, 0.75, 0.80, 0.85 and 0.91. The mobile phase chloroform:acetone (7.5:2.5) resolved 9 spots with R*f* values 0.04, 0.06, 0.13, 0.24, 0.28, 0.41, 0.48, 0.57 and 0.91. The minimum number of 6 spots was observed in mobile phase of chloroform alone (10:0) with R*f* values 0.06, 0.10, 0.15, 0.33, 0.41 and 0.91 (Plate 1; Tables 1, 2, 3).

Rashed et al (2019) reported that the TLC of methanol extract of *Moringa oleifera* leaves revealed the presence of four compounds with R*f* values 0.01, 0.89, 0.92 and 0.93 with the solvent phase [chloroform:methanol:ethanol (1:1:1)]. With ethanol

Table 1. R*f* values of chloroform extract of *P corylifolia* through thin layer chromatography (TLC) in chloroform:methanol (09:01) solvent system

Band number	Colour of the band	Distance travelled (cm)	R*f* value
B1	Pale green	0.5	0.04
B2	Light blue	1.5	0.12
B3	Brown	2.0	0.16
B4	Light green	2.8	0.23
B5	Golden yellow	3.0	0.25
B6	Yellow	3.6	0.30
B7	Orange	4.0	0.33
B8	Pale yellow	5.3	0.44
B9	Dark green	6.0	0.50
B10	Olive green	6.5	0.54
B11	Purplish brown	7.4	0.61
B12	Light brown	8.0	0.66
B13	Bluish green	8.5	0.70
B14	Ash violet	9.0	0.75
B15	Green	9.6	0.80
B16	Dark brown	10.2	0.85
B17	Golden yellow	11.0	0.91



Chloroform (10:0) **Chloroform:methanol (09:1)** **Chloroform:acetone (7.5:2.5)**

Plate 1. TLC for chloroform leaf extract of *Psoralea corylifolia*

Table 2. R*f* values of chloroform extract of *P corylifolia* through thin layer chromatography (TLC) in chloroform (10:0) solvent system

Band number	Colour of the band	Distance travelled (cm)	R*f* value
B1	Green	0.8	0.06
B2	Light yellow	1.2	0.10
B3	Deep yellow	1.8	0.15
B4	Ash violet	4.0	0.33
B5	Orange	5.0	0.41
B6	Golden yellow	11.0	0.91

Table 3. R*f* values of chloroform extract of *P corylifolia* through thin layer chromatography (TLC) in chloroform:acetone (7.5:2.5) solvent system

Band number	Colour of the band	Distance travelled (cm)	R*f* value
B1	Light Green	0.5	0.04
B2	Dark green	0.8	0.06
B3	Yellowish green	1.6	0.13
B4	Yellow	2.9	0.24
B5	Pale brown	3.4	0.28
B6	Ash violet	5.0	0.41
B7	Green	5.8	0.48
B8	Olive green	6.9	0.57
B9	Golden yellow	11.0	0.91

extract of the same solvent phases, maximum of 5 bands with R*f* values 0.046, 0.66, 0.83, 0.90 and 0.93 were recorded. Singh and Panda (2005) reported that the active compounds like carvacrol, new diterpenes, coleonol D, coleol, coleonone, coleonol E, coleonol, forsicolin, crocetin dialdehyde, barbatusol and plectin were found in the essential oil extracted from roots of *Plectranthus amboinicus*.

Mixture of solvents with variable polarity in different ratios can be used for separation of pure compound from the plant extract. The selection of appropriate solvent system for a particular plant extract can be obtained by analyzing the R*f* values of compounds in different solvent system (Sharma et al 2011).

REFERENCES

Manilal A, Sujith S, Sabarathnam B, Kiran SG, Selvin J, Shakir C and Lipton AP 2010. Bioactivity of the red algae *Asparagopsis taxiformis* collected from the southwestern coast of India. Brazilian Journal of Oceanography **58(2):** 93-100.

Rajauria G and Abu-Ghannam N 2013. Isolation and partial characterization of bioactive fucoxanthin from *Himanthalia elongata* brown seaweed: a TLC-based approach. International Journal of Analytical Chemistry **2013:** 802573; doi: 10.1155/2013/802573.

Rashed A, Shaeroun A, Ahmed AB, Alqamoudy H, Mohamed KS, Almunir N, Kushlaf N, El-Mahmoudy, Misbah AA, Oshkondali STM and Almesai ZR 2019. Thin layer chromatography (TLC) and phytochemical analysis of *Moringa oleifera* methanol, ethanol, water and ethyl acetate extracts. Saudi Journal of Medical and Pharmaceutical Sciences **5(10):** 817-820.

Sadasivam S and Manickam A 2005. Biochemical methods. 2nd edn, New Age International (P) Limited Publishers, New Delhi, India.

Sharma AK, Dutt D, Upadhyaya JS and Roy TK 2011. Anatomical, morphological and chemical characterization of *Bambusa tulda*, *Dendrocalamus hamiltonii*, *Bambusa balcooa*, *Malocana baccifera*, *Bambusa arundinacea* and *Eucalyptus tereticornis*. BioResources **6(4):** 5062-5073.

Singh SM and Panda AK 2005. Solubilization and refolding of bacterial inclusion body proteins. Journal of Bioscience and Bioengineering **99(4):** 303-310.

Swaroop MR, Sathyanarayana BD, Vasudevan P, Aneesa, Kumari P and Raghavendra J 2016. Evaluation of efficacy and safety of modified technique of auto wart implantation in the treatment of multiple, recurrent and recalcitrant warts. Indian Journal of Clinical and Experimental Dermatology **2(1):** 27-31.

Urzua A, Echeverría J, Rezende MC and Wilkens M 2008. Antibacterial properties of 3 H-Spiro [1-benzofuran-2, 1'-cyclohexane] derivatives from *Heliotropium filifolium*. Molecules **13(10):** 2385-2393.

Vijayabaskar P and Shiyamala V 2011. Antibacterial activities of brown marine algae (*Sargassum wightii* and *Turbinaria ornata*) from the Gulf of Mannar Biosphere Reserve. Advances in Biological Research **5(2):** 99-102.

Zhang X, Zhao W, Wang Y, Lu J and Chen X 2016. The chemical constituents and bioactivities of *Psoralea corylifolia* Linn: a review. American Journal of Chinese Medicine **44(1):** 35-60.