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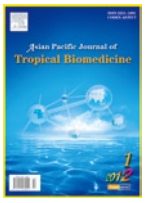

Document heading

High performance thin layer chromatography profile studies on the alkaloids of *Albizia lebbeck*

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ABSTRACT

Objective: To establish alkaloids profile for the medicinally important plant *Albizia lebbeck* (*A. lebbeck*) using high performance thin layer chromatography (HPTLC). **Methods:** Preliminary phytochemical screening was carried out by Harborne method. HPTLC studies were followed by Harborne and Wagner *et al* method. Ethyl acetate–methanol–water (100:13.5:10) was employed as mobile phase for alkaloids. The developed plate was sprayed with Dragendorff's reagent followed by 10% sodium nitrite reagent as spray reagent and dried at 100 ℃ in hot air oven for 3 min. The plate was photo-documented at UV 366 nm and daylight using photo-documentation chamber. **Results:** Alkaloids, steroids, terpenoids, flavonoids, saponins and glycosides present in the petroleum ether, ethyl acetate and methanolic extracts of *A. lebbeck* leaves. The petroleum ether extracts of *A. lebbeck* leaves displayed the presence of 10 types of alkaloids with 10 different Rf values ranging from 0.02 to 0.85. The ethyl acetate extract of *A. lebbeck* leaves illustrated the presence of 5 different types of alkaloids with 5 different Rf values ranging from 0.09 to 0.84. The methanolic extract of *A. lebbeck* leaves demonstrated the presence of 4 different types of alkaloids with 4 different Rf values with range from 0.02 to 0.79. Maximum number (10) of alkaloids has been observed in petroleum ether followed by ethyl acetate (5) extracts of the leaves of *A. lebbeck*. **Conclusions:** In the present study we observed various alkaloids profile of the medicinally important plant using HPTLC. This profile can be used for the identification of the medicinally important plants from the adulterant. Further, separation and characterization of the bioactive compound (principles) from the plants is to be evaluated and reported in near future.

1. Introduction

Plants generally contain primary and secondary metabolites namely alkaloids, terpenoids, flavonoids, saponins, coumarins, glycosides, phenolics, carboxylic acids, aminoacids, sugars, proteins etc. These phyto-constituents impart the specific characteristics and properties of plants. Therefore, it is obligatory to resolve all of the phytochemical constituents present in the plants in order to ensure the consistency and repeatability of pharmacological, antimicrobial and clinical research, to understand their bioactivities, identify the active principles (components) and possible side effects of active compounds and to enhance product quality control[1]. These phyto-constituents are estimated quantitatively and qualitatively by a variety of techniques such as spectroscopy and chromatography. Chromatography techniques are the most useful and popular tools used for the qualitative and separation studies. High performance thin layer chromatography (HPTLC) chromatographic fingerprints can be applied for this kind of certification. Finger print analysis by HPTLC has developed into an effective and powerful tool for linking the chemical constituents' profile of the plants with botanical identity and for estimation of chemical and biochemical markers[2-10]. *Albizia lebbeck* (*A. lebbeck*) Benth is widely distributed in India and is also found in South Africa and Australia. Traditionally, the barks are used in toothache and diseases of the gum. Decoction of the leaves and barks are protective against bronchial asthma and other allergic disorders. Barks and seeds are astringent and are given in piles and diarrhea.

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Ethanolic and methanolic extracts of pods possesses anti-protozoal, anti-fertility activity, hypoglycemic and anticancer properties[11-14]. The plant extract is reported to have antiseptic, anti- dysenteric, anti-ovulatory, nootropic, anti-inflammatory, antimicrobial activity and anti-tubercular activities[15-18]. The plant also contains saponins, macrocyclic alkaloids, anthraquinone glycosides, tannins, and flavonols[17]. The saponin constituents of *Albizia* so far described are echinocystic acid glycosides[19,20]. The albiziasaponins A, B, and C were isolated from the barks of *A. lebbeck*[21]. Phytochemical investigations of *A. lebbeck* pod showed that they contains 3′, 5 Dihydroxy 4′, 7 dimethoxy flavone, and N-benzoyl L phenylalaninol[22]. The beans of the plant contain albigenic acid-a new triterpenoid sapogenin[23]. The tri-O-glycoside flavonols kaempferol and quercetin were identified from the leaves of *A. lebbeck*[24]. Albizzia-hexoside a new hexaglycosylated saponin was isolated from leaves of *A. lebbeck*[25]. Misra *et al*[26] isolated N-demethyl budmunchiamines from *A. lebbeck* seeds and Maa *et al*[27] confirmed the tannin presence in *A. lebbeck*. With this knowledge the present study was aimed to produce the alkaloids HPTLC profile for the medicinally important plant *A. lebbeck*.

2. Materials and methods

A. lebbeck was collected from natural habitats, Rasipuram, Nammakkal, Tamil Nadu, India, and authenticated by Dr. EG Wesely and the specimens voucher were deposited in the St. Xavier's College Herbarium for further reference. The fresh leaves were shade dried and powdered using the electric homogenizer. The powdered samples were extracted with 150 mL of petroleum ether, methanol and ethyl acetate for 8-12 h by using the soxhlet apparatus. Preliminary phytochemical screening was done by following the method of Harborne[28], HPTLC studies were carried out following Harborne[29] and Wagner *et al*[30]. For the present study CAMAG HPTLC system equipped with Linomat V applicator, TLC scanner 3, Reprostar 3 with 12 bit CCD camera for photo documentation, controlled by WinCATS- 4 software were used. All the solvents used for HPTLC analysis was obtained from MERCK. The samples (5 μL) were spotted in the form of bands of width 5 mm with a Camag microlitre syringe on pre-coated silica gel glass plate 60F-254 (20 × 10 cm with 250 μm thickness (E. Merck, Darmstadt, Germany) using a Camag Linomat IV (Switzerland). The plates were pre-washed by methanol and activated at 60 ℃ for 5 min prior to chromatography. The sample loaded plate was kept in TLC twin trough developing chamber (after saturated with solvent vapor) with respective mobile phase (alkaloids) and the plate was developed in the respective mobile phase up to 90 mm. The ethyl acetate-methanol-water (100: 13.5: 10) was employed as mobile phase for alkaloids. Linear ascending development was carried out in 20 cm × 10 cm twin trough glass chamber (Camag, Mutenz, Switzerland) saturated with the mobile phase and the chromatoplate development for two times with the same mobile phase to get good resolution of phytochemical contents. The optimized chamber saturation time for mobile phase was 30 min at room temperature (25 ± 2) ℃. The developed plate was dried by hot air to evaporate solvents from the plate. The developed plate was sprayed with Dragendorff's reagent followed by 10% sodium nitrite reagent as spray reagent and dried at 100 ℃ in hot air oven for 3 min. The plate was photo-documented at UV 366 nm and daylight using photo-documentation (CAMAG REPROSTAR 3) chamber. Finally, the plate was fixed in scanner stage and scanning was done at 366 nm. The plate was kept in photo-documentation chamber (CAMAG REPROSTAR3) and captured the images under white light, UV light at 254 and 366 nm. Densitometric scanning was performed on Camag TLC scanner III and operated by CATS software (V 3.15, Camag).

3. Results

The results of the preliminary phytochemical studies confirm the presence of alkaloids, steroids, terpenoids, flavonoids, saponins and glycosides in the petroleum ether, ethyl acetate and methanolic extracts of *A. lebbeck* leaves. Different compositions of the mobile phase for HPTLC analysis were tested in order to obtain high resolution and reproducible peaks. The aim was achieved using ethyl acetate-methanol-water (100: 13.5: 10) as the mobile phase (Figure 1 a-d). The petroleum ether extracts of *A. lebbeck* leaves displayed the presence of 10 types of alkaloids with 10 different Rf values with range from 0.02 to 0.85 (Table 1). The ethyl acetate extract of *A. lebbeck* leaves illustrated the presence of 5 different types of alkaloids with 5 different Rf values with range from 0.09 to 0.84 (Table 2). The methanolic extract of *A. lebbeck* leaves demonstrated the presence of 4 different types of alkaloids with 4 different Rf values with range from 0.02 to 0.79 (Table 3). Maximum number (10) of alkaloids has been observed in petroleum ether followed by ethyl acetate (5) extracts of the leaves of *A. lebbeck*.

Table 1

HPTLC – Alkaloid profile of the petroleum ether extracts of *A. lebback*.

Track	Peak	Rf	Height	Area	Assigned substance
A	1	0.02	174.8	2742.2	Unknown
A	2	0.13	126.8	2497.6	Unknown
A	3	0.20	54.5	1095.7	Unknown
A	4	0.24	26.8	476.9	Unknown
A	5	0.36	14.5	366.9	Unknown
A	6	0.44	16.8	444.0	Unknown
A	7	0.62	15.9	348.6	Unknown
A	8	0.75	264.0	6440.4	Unknown
A	9	0.78	475.0	13119.3	Unknown
A	10	0.85	347.8	17548.4	Unknown

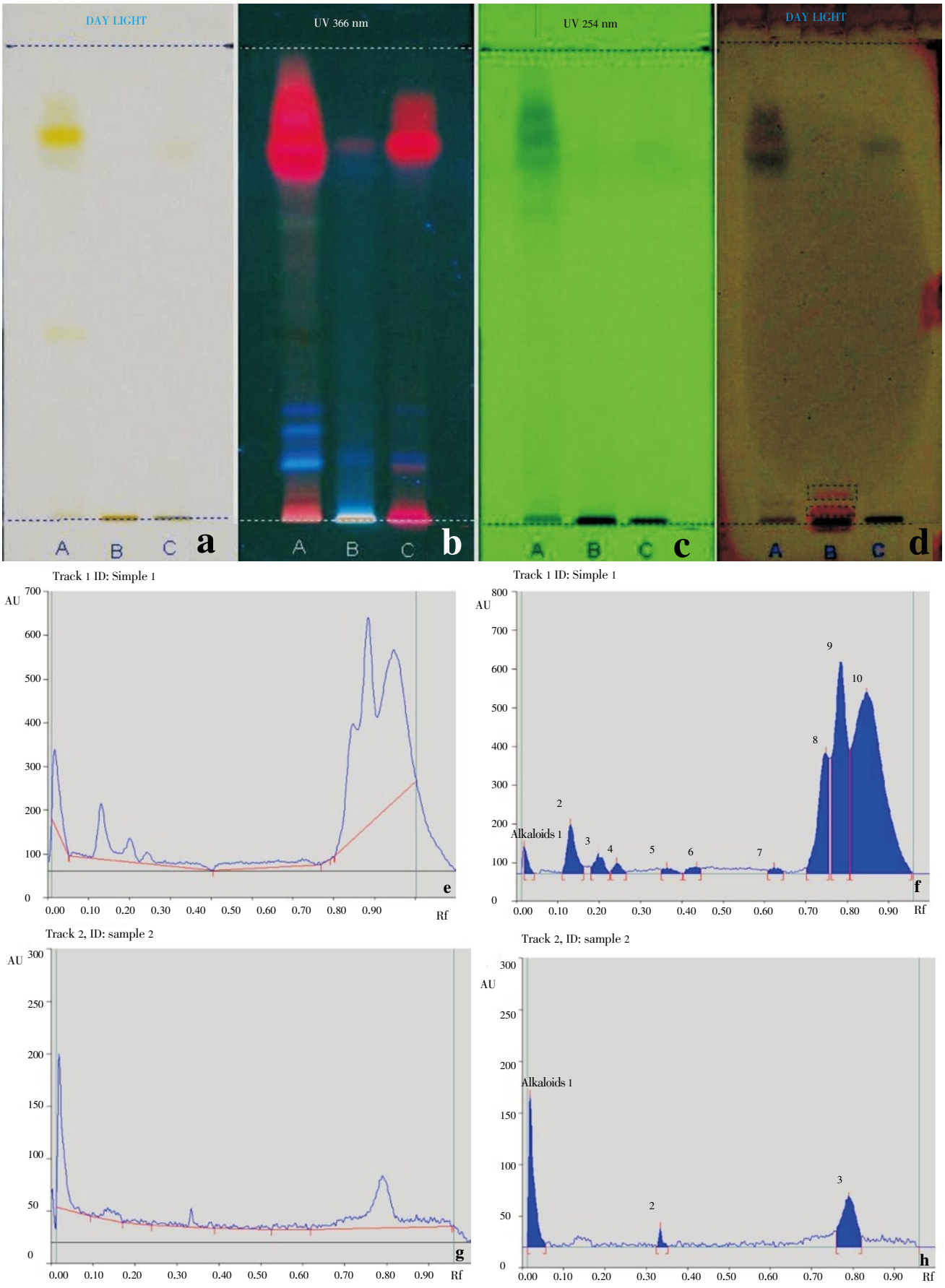

Figure 1. a) HPTLC profile of the *A. lebbeck* under daylight; b) HPTLC profile of the *A. lebbeck* under UV 366; c) HPTLC profile of the *A. lebbeck* under UV 254; d) HPTLC profile of the *A. lebbeck* under day light – after derivation; e) HPTLC chromatogram of petroleum ether extracts of *A. lebbeck* – baseline display; f) HPTLC chromatogram of petroleum ether extracts of *A. lebbeck* – peak densitogram display; g) HPTLC chromatogram of methanolic extracts of *A. lebbeck* – baseline display; h) HPTLC chromatogram of methanolic extracts of *A. lebbeck* – peak densitogram display. All scanned at 366 nm.

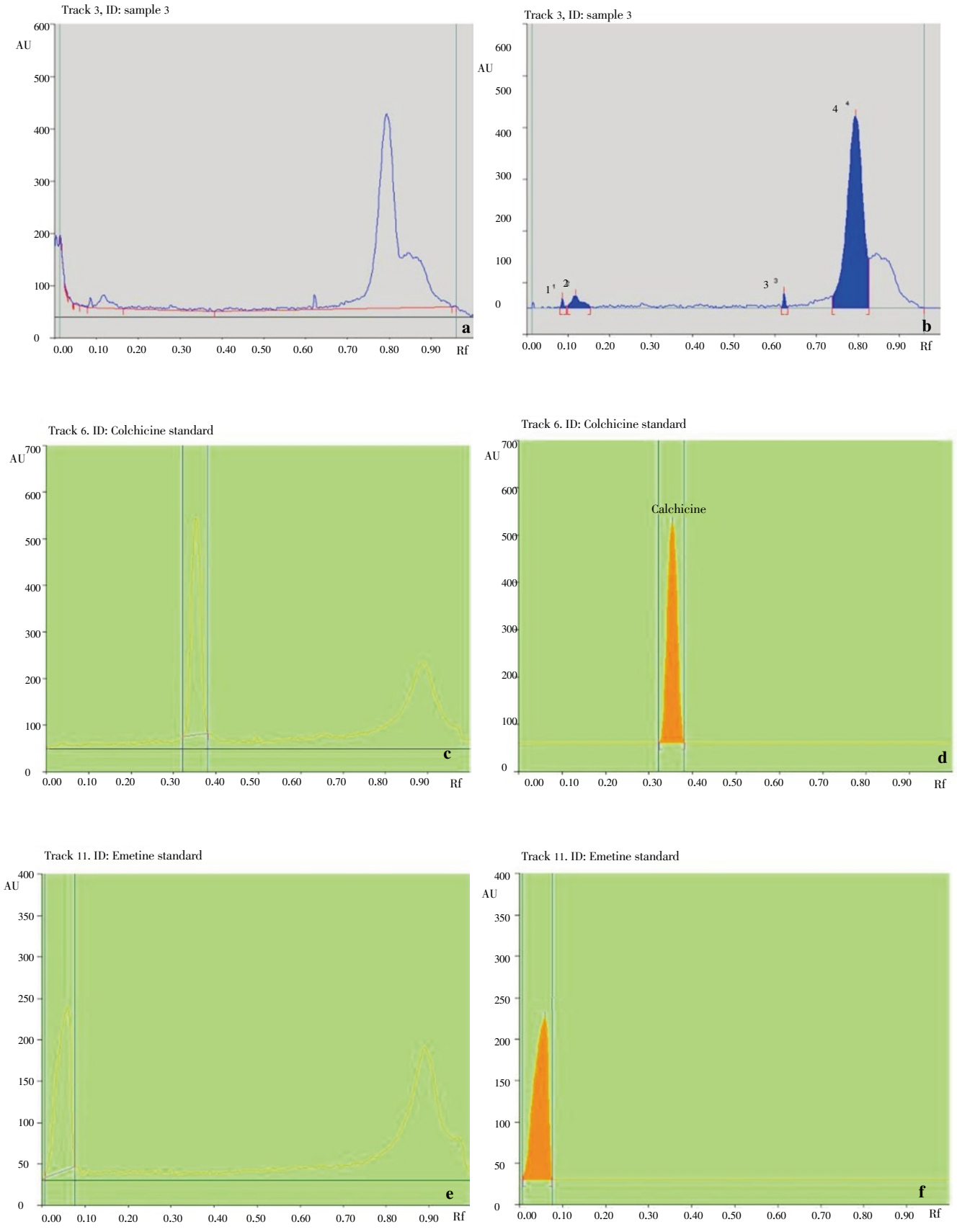

Figure 2. a) HPTLC chromatogram of ethyl acetate extracts of *A. lebbeck* – baseline display – scanned at 366 nm; b) HPTLC chromatogram of ethyl acetate extracts of *A. lebbeck* – peak densitogram display – scanned at 366 nm; c) HPTLC chromatogram of standard colchicine – scanned at 366 nm; d) HPTLC chromatogram of standard colchicine peak densitogram display – scanned at 366 nm; e) HPTLC chromatogram of standard emetine – scanned at 366 nm; f) HPTLC chromatogram of standard emetine peak densitogram display – scanned at 366 nm.

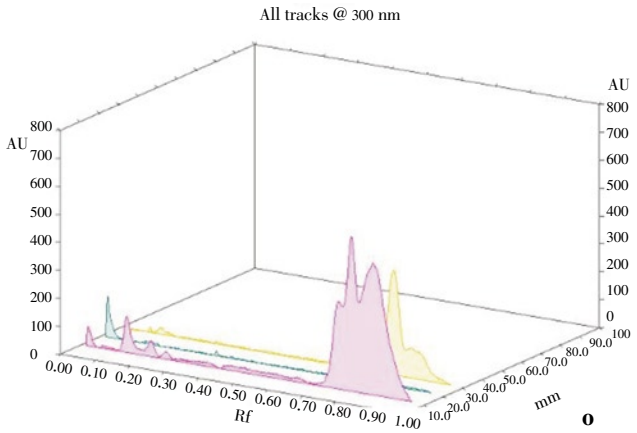

Figure 3. 3D display of HPTLC chromatogram of *A. lebbeck* – petroleum ether, methanolic and ethyl acetate leaves extracts of *A. lebbeck*.

Table 2

HPTLC – Alkaloid profile of the methanolic extracts of *A. lebback*.

Track	Peak	Rf	Height	Area	Assigned substance
B	1	0.02	166.9	2341.4	Alkaloid 1
B	2	0.14	20.2	556.7	Unknown
B	3	0.33	20.2	186.0	Unknown
B	4	0.79	47.4	1476.5	Unknown

Table 3

HPTLC – Alkaloid profile of the ethyl acetate extracts of *A. lebback*.

Track	Peak	Rf	Height	Area	Assigned substance
C	1	0.09	18.8	100.5	Unknown
C	2	0.12	24.5	564.4	Unknown
C	3	0.62	27.2	139.3	Unknown
C	4	0.79	354.0	11949.0	Unknown
C	5	0.84	82.6	3416.0	Unknown

Table 4

HPTLC – Alkaloid profile of *A. lebback* leaves extracts.

Rf	Petroleum ether	Methanol	Ethyl acetate
0.02	+	+	–
0.09	–	–	+
0.12	–	–	+
0.13	+	–	–
0.14	–	+	–
0.20	+	–	–
0.24	+	–	–
0.33	–	+	–
0.36	+	–	–
0.44	+	–	–
0.62	+	–	+
0.75	+	–	–
0.78	+	–	–
0.79	–	+	+
0.84	–	–	+
0.85	+	–	–

The well resolved HPTLC profile of the methanolic, ethyl acetate and petroleum ether extracts of *A. lebbeck* leaves were depicted in Figure 1a–d and the Rf value, height and area were recorded in Table 1 to 3 to validate the presence of alkaloids in leaves of *A. lebbeck*. HPTLC chromatogram of the standard colchicines, emetine and the alkaloid profile (Table 4) of *A. lebbeck* are illustrated in Figure 1e–h, Figure 2 and Figure 3.

4. Discussion

Secondary metabolites are produced by a large variety of organisms, including bacteria, fungi, plants and animals especially by higher plants for their defensive mechanisms to protect themselves from the biotic and abiotic factors. Of these secondary metabolites about 10 to 25% are alkaloids and are part of the group of natural products. Alkaloids possess lots of pharmacological and pharmaceutical properties and are used as medicines, as recreational drugs, or in entheogenic rituals[31]. The quality and quantity of the alkaloids present in the plants are varied depending on the type of plants and parts or tissue of the plants. Highest concentration is observed in the leaves (black henbane), fruits or seeds (strychnine tree), root (*Rauwolfia serpentina*) or bark (cinchona)[31]. Similar to the previous observations, in the present study we also observed different alkaloids profiles with various Rf values, intensity, area, height in the leaves extracts of *A. lebbeck*. In addition we observed the variation in the alkaloids profile based on the solvents used for the extraction (Table 1–5). The medicinal usage and application of alkaloid plants has a long history, and thus when the first alkaloids were synthesized in the 19th century, they immediately found application in clinical practice[29]. Many alkaloids are still used in medicine, usually in the form of salts, including the following: anti-arrhythmic, anti-cholinergic, anti-tumor, vasodilating, anti-hypertensive, cough medicine, anesthetic, anti-microbial, anti-protozoal agent. The results of the present study authenticates and confirms the folkloric usage, traditional practices, ethnobotanical, anti-microbial and pharmacological values of the medicinally important plant *A. lebbeck* and suggest that the leaves extracts of *A. lebbeck* possess compounds with bioactivity properties that can be used as active principles or agents in new drugs for the therapy of infectious diseases. A recent review proves that the HPTLC techniques can be used to rectify many qualitative and quantitative analytical problems in a wide range of fields including medicines, pharmaceutical, chemistry, biochemistry and toxicology[31]. In addition, HPTLC was recommended for identification of the medicinal plants and finds solution for the taxonomical problems[8-10]. Similar to the previous observations, in the present study we produced the HPTLC profile for the methanolic, ethyl acetate and petroleum ether extracts of

A. lebbeck leaves. This can be used in the pharmaceutical industry as a pharmacogonstical tool to identify this medicinally important plant. In addition it can be adopted as a chemo-taxonomical tool in the plant systematic. Further, the separation and characterization of the bioactive compound (principles) from the plants is to be evaluated and reported in near future.

Conflict of interest statement

We declare that we have no conflict of interest.

References

[1] Amani M, El-Mousallamy D. Leaf flavonoids of *Albizia lebbeck*. *Phytochemistry* 1998; **48**(4): 759 -761.

[2] Dash S, Das C, Sahoo DC. Phytochemical and anthelmintic screening of crude bark extract of *Adenanthera pavonina* Linn. *Int J Compr Pharm* 2010; **2**(10): 1-4.

[3] Patil AG, Koli SP, Patil DA, Chandra N. Pharmacognostical standardization and HPTLC fingerprint of *Crataeva tapia* Linn. SSP. Odora (Jacob.) Almeida leaves. *Int J Pharm Biosci* 2010; **1**(2): 1-14.

[4] Ramya V, Dheena Dhayalan V, Umamaheswari S. *In vitro* studies on antibacterial activity and separation of active compounds of selected flower extracts by HPTLC. *J Chem Pharm Res* 2010; **2**(6): 86-91.

[5] Tripathi AK, Verma RK, Gupta AK, Gupta MM, Khanuja PSS. Quantitative determination of phyllanthin and hypophyllanthin in phyllanthus species by high-performance thin layer chromatography. *Phytochem Anal* 2006; **17**: 394-397.

[6] Manikandan A, Victor Arokia Doss A. Evaluation of biochemical contents, nutritional value, trace elements, SDS-PAGE and HPTLC profiling in the leaves of *Ruellia tuberosa* L. and *Dipteracanthus patulus* (Jacq.). *J Chem Pharm Res* 2010; **2**(3): 295-303.

[7] Sharma RJ, Chaphalkar SR, Adsool AD. Evaluating antioxidant potential, cytotoxicity and intestinal absorption of flavonoids extracted from medicinal plants. *Int J Biotechnol Appl* 2010; **2**(1): 01-05.

[8] Yamunadevi M, Wesely EG, Johnson M. Chromatographic finger print analysis of steroids in *Aerva lanata* L by HPTLC technique. *Asian Pac J Trop Biomed* 2011; **1**: 428-433.

[9] Yamunadevi M, Wesely EG, Johnson M. Chemical profile studies on the alkaloids of medicinally important plant *Aerva lanata* L. using HPTLC. *J Nat Conscientia* 2011; **2**(2): 341-349.

[10] Yamunadevi M, Wesely EG, Johnson M. A chromatographic study on the glycosides of *Aerva lanata* L. *Chin J Nat Med* 2011; **9**(3): 210-214.

[11] Watt JM, Breyer-Brandwijk MG. *The medicinal and poisonous plants of south and east africa*. 2nd ed. Edinburgh: Livingstone; 1962.

[12] Chadha YR, editor. *The wealth of India*. New Delhi: CSIR, Raw Materials; 1985.

[13] Gupta RS, Kachhawa JB, Chaudhary R. Anti-fertility effects of methanolic pod extract of *Albizia lebbeck* Benth. in male rats. *Asian J Androl* 2004; **6**(2): 155-159.

[14] Gupta RS, Chaudhary R, Yadav RK, Verma SK, Dobhal MP. Effect of saponins of *Albizia lebbeck* Benth. bark on the reproductive system of male albino rats. *J Ethnopharmacol* 2005; **96**(1-2): 31-36.

[15] Chintawar SD, Somani RS, Kasture VS, Kasture SB. Nootropic activity of *Albizia lebbeck* in mice. *J Ethnopharmacol* 2002; **81**(3): 299-305.

[16] Pratibha N, Saxena VS, Amit A, D'Souza P, Bagchi M, Bagchi D. Anti-inflammatory activities of Aller-7, a novel polyherbal formulation for allergic rhinitis. *Int J Tissue React* 2004; **26**(1-2): 43-51.

[17] Chulet R, Pradhan P, Sharma KS, Jhajharia KM. Phytochemical screening and antimicrobial activity of *Albizzia lebbeck*. *J Chem Pharm Res* 2010; **2**(5): 476-484.

[18] Kapoor BBS, Bhumika, Khatri JS. Antimicrobial activity of some medicinal tree species of Hanumangarh district of Rajasthan. *J Phytological Res* 2007; **20**: 325-326.

[19] Carpani G, Orsini F, Sisti M, Verotta L. Saponin forms *Albizia antheimintica*. *Phytochemistry* 1989; **28**: 863-866.

[20] Orsini F, Pelizzoni F, Verotta L. Saponins from *Albizia lucida*. *Phytochemistry* 1991; **30**(12): 4111-4115.

[21] Pal BC, Achari B, Yoshikawa K, Arihara S. Saponins from *Albizia lebbeck*. *Phytochemistry* 1995; **38**(5): 1287-1291.

[22] Rashid RB, Chowdhury R, Jabbar A, Hasan CM, Rashid MA. Constituents of *Albizia lebbeck* and antibacterial activity of an isolated flavone derivatives. *Saudi Pharm J* 2003; **11**(1-2): 52-56.

[23] Barua AK, Raman SP. The constitution of albigenic acid-A new triterpenoid sapogenin from *Albizia lebbeck* Benth. *Tetrahedron* 1959; **7**: 19-23.

[24] El-Mousallamy AMD. Leaf flavonoids of *Albizia lebbeck*. *Phytochemistry* 1998; **48**(4): 759-761.

[25] Ueda M, Tokunaga T, Okazaki M, Sata NU, Ueda K, Yamamura S. Albiziahexoside: A potential source of bioactive saponin from the leaves of *Albizzia lebbeck*. *Nat Prod Res* 2003; **17**(5): 329-335.

[26] Misra LN, Dixit AK, Wagner H. N-demethyl budmunchiamines from *Albizia lebbeck* seeds. *Phytochemistry* 1995; **39**(1): 247-249.

[27] Maa YT, Hsiaob SC, Chenb HF, Hsu FL. Tannins from *Albizia lebbeck*. *Phytochemistry* 1997; **46**(8): 1451-1452.

[28] Harborne JB. *Phytochemical methods*. London: Chapman and Hall; 1973.

[29] Harborne JB. *Phytochemcial methods*. 3rd ed. London: Chapman and Hall; 1998;

[30] Wagner H, Baldt S, Zgainski EM. *Plant drug analaysis*. Berlin: Springer; 1996.

[31] David FR. Evolution of plant chemical defense against herbivores. In: Gerald AR, Daniel HJ. *Herbivores: Their interaction with secondary plant metabolites*. New York: Academic Press; 1979.