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Research Article

Chromatographic finger print analysis of budmunchiamines in *Albizia amara* by HPTLC technique

T. Rajkumar* and B.N.Sinha

Department of Pharmaceutical Sciences,Birla Institute of Technology, Mesra, Ranchi-835215, Jharkhand, India

ABSTRACT

Recently, there has been an increase in the use of herbal drugs for healthcare. Herbal Medicine is a time-tested and valuable resource for healing. In India, the herbal drug market is about $ one billion and the export of plant based crude drugs is around $ 80 million. So it is absolutely essential to provide scientifically proven and evidence based herbal formulations for global acceptance. Chromatographic fingerprint symbolize the active chemical constituents of herbal medicines for desired therapeutic action. This study presents a simple, rapid and selective HPTLC method for separation and determination of *Albizia amara*. *Albizia amara* (Fabaceae) is the medicinal plant which has been used to treat piles, diarrhoea, gonorrhoea, leprosy, and leucoderma. The macro cyclic alkaloid Budmunchiamines is the main constituent which has been separated and identified by camag HPTLC. The coarsely dried powdered leaves were extracted with Petroleum Ether (60-80)^{0}C by cold maceration, and hot percolation with 90% methanol for 72 h. TLC profile and HPTLC fingerprints were recorded by using silica gel GF_{254} as stationary phase. Separation was performed on pre-coated 10x10cm HPTLC Aluminium sheets. Sample volumes of (5µl) were applied to the 6 tracks contain bandwidth of 5mm and 10 secs/µl using a TLC applicator. The plates were developed using a mobile phase of Chloroform: diethylamine (86:22 v/v). The peak 2, 6 reveals that the presence of macro cyclic alkaloids Budmunchiamines L4, L5 were characterised and confirmed by FTIR, ^{1}H NMR, MASS and CHN analysis. The results were compared with earlier reports. The developed HPTLC fingerprints will help the manufacturer for quality control and standardization of herbal formulations.

Keywords: Budmunchiamines; HPTLC; Fingerprint; *Albizia amara*.

INTRODUCTION

Herbal drugs have been in use by different civilizations in different parts of the world for centuries to fight a large number of diseases. Many of these are in common use even today. *Albizia* is a genus of about 150 species of mostly fast-growing subtropical and tropical trees and shrubs in the Subfamily Mimosoideae of the legume family, Fabaceae. The genus is pantropical, occurring in Asia, Africa, Madagascar, Central, South, and southern North America and Australia, but mostly in the Old World tropics. Some species are considered weedy. They are commonly called silk trees or sirises (*Albizia* species, [Online]). *Albizia amara* is a small to moderate-sized, much-branched deciduous tree with smooth, dark green, scaly bark. It resembles the acacias but lacks thorns. Its root system is shallow and spreading. The leaves are pinnately compound, with 15-24 pairs of small, linear leaflets, on 6-15 pairs of pinnate. The yellow, fragrant and globose flowers are in clusters. They develop when the tree is almost leafless. Flowers pedicelled, yellow, fragrant, in 12-20 globose heads. Fruits are oblong pods, about 10-28 x 2-5 cm, light brown, puberulous, thin, and 6-8 seeded; seeds flattened, 8-13 x 7-8 mm (Orwa *et al*., 2009). The seeds of *Albizia amara* (Fabaceae) used as an astringent, treating piles, diarrhoea, gonorrhoea, leprosy, leucoderma, erysipelas and abscesses. The leaves and flowers have been applied to boils, eruptions, and swellings, also regarded as an emetic and as a remedy for coughs, ulcer, dandruff and malaria (Woongchon *et al*.,1991). A lot of analytical techniques have been developed for renewed quality control of herbal formulation drugs from plant origin. Chromatographic fingerprinting of phyto constituents can be used for the assessment of quality consistency and stability of herbal extracts or products by visible observation and comparison of the standardized fingerprint pattern. So an attempt has been made to develop a HPTLC chromatograms and its finger print characterization for the qualitative and quantitative work.

The fingerprint has potential to determine authenticity and reliability of chemical constituents of herbal drug and formulations. *Albizia amara* is among the few medicinal plants by virtue of their extensive medicinal uses. *Albizia amara* has been chosen as the plant to perceive its active chemical constituent's alkaloids,

* Corresponding Author
Email: rajkutty1983@gmail.com
Contact: +91-9199443215
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flavonoids, steroids, phenols and tannins through Chromatographic finger print analysis of High-Performance Thin-Layer Chromatography with UV Spectroscopy.

EXPERIMENTAL

Plant Material

The leaves of *Albizia amara* were procured from medicinal garden of Medicinal plants Revitalisation and Rehabilitation Centre, Sevaiyur, Tamilnadu and authenticated by Dr.S.Jha, Professor, Birla Institute of Technology, Mesra, Ranchi, India. The authenticated specimen has been deposited (PHARM/HS/14/09-10) in the department.

Sample preparation and extraction

The crude drugs were dried under shade for 4-6 days. Then the dried materials were milled to powder. This powdered material was again dried in the oven at 40 ^{0} C for 4 h and used for extraction followed by concentration, screening, TLC, HPTLC and column chromatography of extracts.

The coarsely powdered dried leaves were extracted with Petroleum Ether (BP-60^0-80^0) cold maceration for 72 h, and hot percolation with 90% methanol about 72 h. Both extracts were recovered and concentrated to dryness.

The Methanolic extract was dissolved in 2% aqueous acetic acid (500 ml) and partitioned with $CHCl_3$, (500 ml). The aqueous acidic fraction was saved, and the $CHCl_3$, fraction was extracted with additional 2% acetic acid (500 ml). The aqueous fractions were combined, adjusted to a pH of 9.0 with Ammonium hydroxide and extracted with $CHCl_3$, (1 litre). The $CHCl_3$, fraction was recovered and concentrated to dryness (Woongchon *et al.*, 1991)

Phytochemical screening

Phytochemical screening of *Albizia amara* were perfomed by standard procedures viz, Test for alkaloids, Test for reducing sugars (Fehling's test), Test for terpenoids (Salkowski test), Test for flavonoids, Test for saponins, Test for tannins (Khandelwal 2007)

Isolation and Purification of Phytoconstituents

Petroleum ether and methanol extracts were subjected to column chromatography using gradient elution technique for the isolation of various phytoconstituents.

HPTLC suggested that 8 compounds were present in Methanolic extract. The MeOH extract was dissolved in 2% aqueous acetic acid (500 ml) and partitioned with $CHCl_3$, (500 ml). The aqueous acidic fraction was saved, and the $CHCl_3$, fraction was extracted with additional 2% acetic acid (500 ml). The aqueous fractions were combined, adjusted to a pH of 9.0 with Ammonium hydroxide and extracted with $CHCl_3$, (1 litre). The $CHCl_3$, fraction was recovered and concentrated to dryness in rotary evaporator.

The basic substance was subjected to Column chromatography (CC) over Silica gel 60-120 mesh (Merck), the spots were visualised by spraying with dragendraff's reagent followed by heating at 100^{0}C for 3 min. column was packed with petroleum ether and eluted by gradient elution technique starting from petroleum ether (60-80^0),chloroform, methanol ,ethyl acetate and water.

$CHCl_3$/MeOH/ 90:10 yielded fraction 8-22 and pooled together with respect to their similar RF values and named Compound SR-01; $CHCL_3$/MeOH (80:20) yielded fraction 23-33 (pooled together, Compound SR-02).

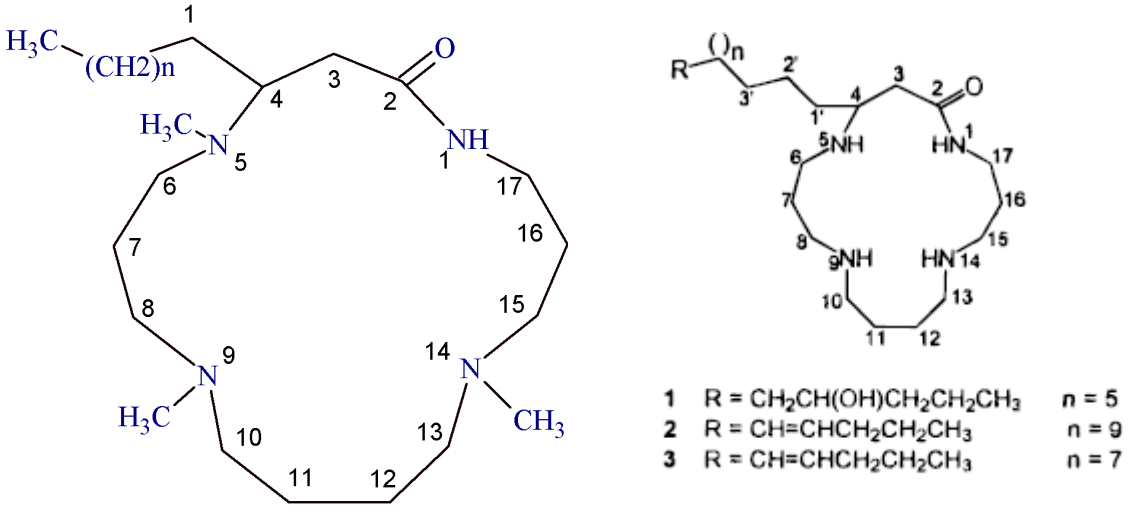

Figure 1: Structures of Budmunchiamines

A. Budmunchiamine **A n = 9**
B. Budmunchiamine **B n = 7**
C. Budmunchiamine **C n = 11**

A. Budmunchiamine **L4**
B. Budmunchiamine **L5**
C. Budmunchiamine **L6**

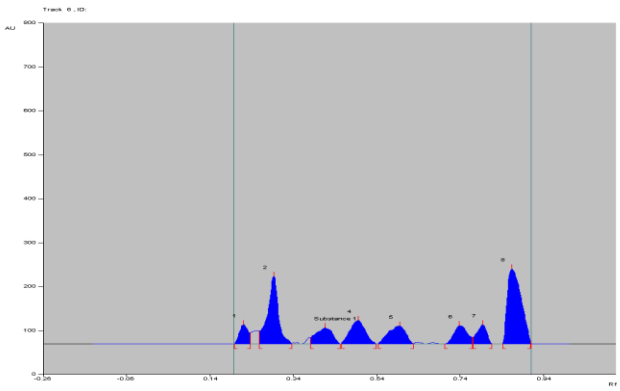

Figure 2: HPTLC Chromatogram of methanolic extract

Table 1: R_f Values of HPTLC analysis of Methanolic Extract

Peak	Rf	Height	Area
1.	0.22	36.9	714.0
2.	0.28	139.9	3178.0
3.	0.42	34.8	1207.7
4.	0.49	52.0	1610.4
5.	0.58	39.1	1370.3
6.	0.73	27.3	596.4
7.	0.79	32.5	631.6
8.	0.86	19.4	5322.4

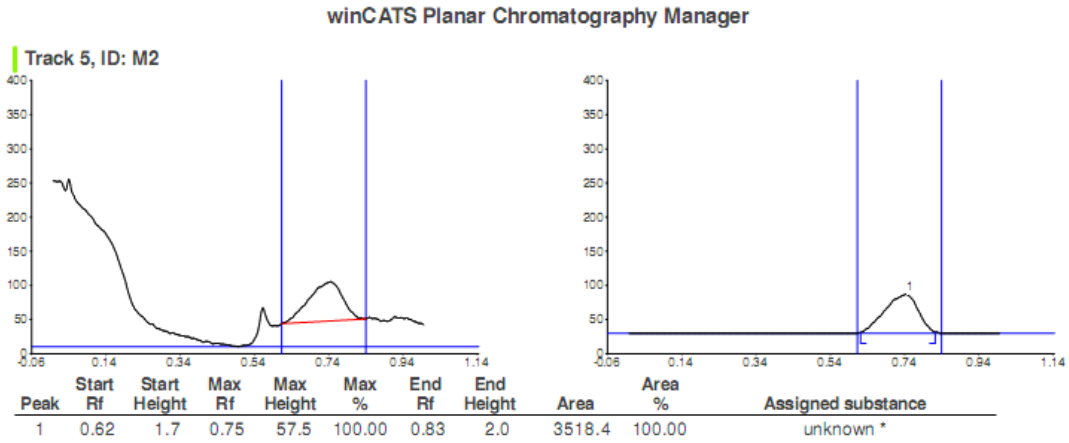

Figure 3: HPTLC Analysis Chromatogram of Compound SR-01

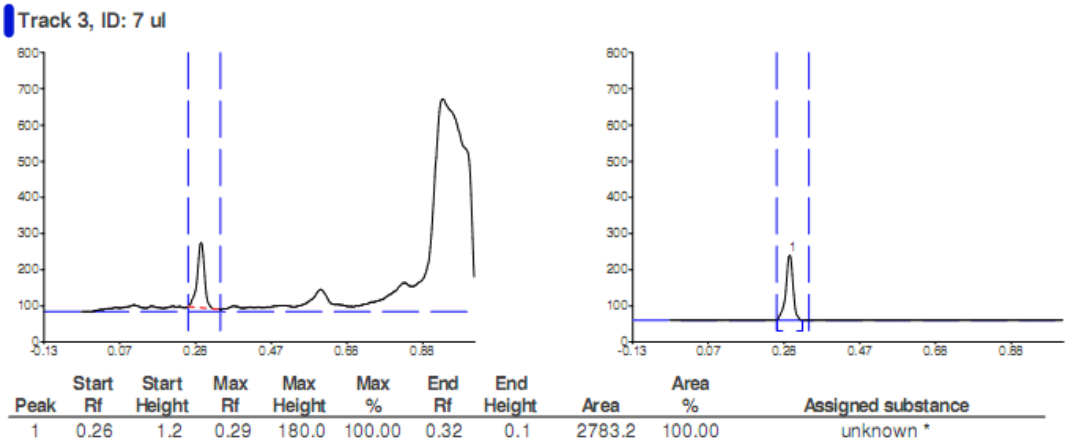

Figure 4: HPTLC Analysis Chromatogram of Compound SR-02

Chromatography

Chromatographic separation of target analyte budmunchiamine from Methanolic extract was performed on 20 cm × 20 cm aluminium-backed HPTLC plates coated with 200 µm layers of silica gel $60GF_{254}$ (Merck, Darmstadt, Germany). Before use, the plates were prewashed with methanol and activated at 110°C for 5 min. Both extracts and isolated compounds (5 µL each) were applied on to HPTLC plate as 6 mm wide bands and 12 mm apart from middle of bands by spray-on technique along with nitrogen gas supply for simultaneous drying of bands, by means of a Camag (Switzerland) Linomat V sample applicator fitted with a 100 µL syringe (Hamilton, Bonaduz, Switzerland). A constant spot application rate of 0.15 µL s-1was used. Plates were developed to a distance of 165 mm, in the dark, with 30 mL chloroform - diethyl amine, 88 : 12 (v / v), as mobile phase. Before development the chamber was saturated with mobile phase for 15 min at room temperature (25 ± 2°C). Chromatography was performed in camag's twin-trough chamber. Wavelength for detection of budmunchiamines was evaluated from complete UV spectrum. (Figure 2). Densitometric scanning was performed with a Camag TLC scanner 3 in reflectance absorbance mode at 236 nm, under control of Camag winCATS planar chromatography manager software (version 1.4.2). The slit dimensions were 5 mm × 0.45 mm and the scanning speed was 10 mm s-1. Chromatogram thus obtained showed peak of budmunchiamines L4 and L5 from crude processed Methanolic extract.

Spectral analysis

TLC profile and HPTLC fingerprint have been developed. The macrocyclic alkaloids Budmunchiamines L4, L5 (Fig. 1) were characterised by UV, FTIR, ^{1}H NMR, Mass and CHN analysis (Woongchon *et al*., 1991, Dixit *et al*., 1997, Rukunga *et al*., 1996, Misra *et al*., 1995). HPTLC fingerprinting and images of the plates were recorded.

RESULTS AND DISCUSSION

From the chromatogram following inferences has been drawn. Peaks 2, 3, 4, 5, 6, 7, 8 were obtained at R_f 0.28, 0.42, 0.49, 0.58, 0.73, 0.79, 0.86 depicts that they must be macrocyclic alkaloids budmunchiamines and other alkaloids

These results of peaks 2, 6 reveals that the presence of macrocyclic alkaloids budmunchiamines L4 and L5 in methanolic extract of *Albizia amara* which has been referred with the earlier reports. But the components of peaks 3, 4, 5, 7, 8 could not be identified due there is no available literature in the background probably would denotes budmunchiamines derivatives. So, we feel that those found in the plant species which need further investigation and exploration is in the pipeline research work.

CONCLUSION

As in the above work it has been revealed that the methanolic extract of *Albizia amara* has 8 peaks in chromatogram which has been produced by HPTLC. Those constituents are macrocyclic alkaloids budmunchiamines L4, L5 which has been referred with the earlier reports. Therefore HPTLC fingerprinting is proved to be a linear, precise, accurate method for herbal formulation and can be used further in quality control of not established herbals. The developed HPTLC fingerprints will help the manufacturer for quality control and standardization of herbal formulations.

REFERENCES

Albizia species. http://en.wikipedia.org/wiki/Albizia. [Accessed:]

Dixit A.K, Misra L.N. Macrocyclic Budmunchiamine Alkaloids from Albizia lebbek, J. Nat. Prod. 1997; 60: 1036-37.

Khandelwal K.R. Practical Pharmacognosy –Techniques and experiments, Nirali Prakasam, 17 th Edn. 2007; 149-61.

Misra L.M, Dixit A.K, Wagner H. n-Demethyl budmunchiamines from *Albizia lebbek* seeds , phytochemistry. 1995; 39(1): 247-49.

Orwa et al, *Albizia amara* botanical information. Agroforestry Database 4.0, 2009; p 2

Rukunga G.M, Waterman P.G. Spermine Alkaloids from *Albizia Schimperana*, Phytochemistry. 1996; 42(4): 1211-15.

Woongchon M, Ghee T.T, Geoffrey A.C, John M.P. Biological activity of novel macrocyclic alkaloids (budmunchiamines) from *Albizia amara* detected on the basis of interaction with DNA. J. Nat. Prod. 1991; 54(6): 1531-42.