IJPSR (2015), Vol. 6, Issue 6 (Research Article)

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 31 October, 2014; received in revised form, 23 December, 2014; accepted, 15 February, 2015; published 01 June, 2015

HIGH PERFORMANCE THIN LAYER CHROMATOGRAPHY OF METHANOLIC BARK EXTRACT FROM *ALBIZIA LEBBECK* LINN.

Ariharasivakumar Ganesan [*1, 2] and Kavimani Subramanian [3]

KMCH College of Pharmacy [1], Coimbatore- 641048, Tamil Nadu, India
Department of Pharmacy, Mewar University, Chittorgarh [2], Rajasthan, India
Mother Theresa Post Graduate and Research Institute of Health Sciences, Puducherry- 605006, Tamil Nadu India

Keywords:

Albizia lebbeck, HPTLC, Diosgenin

Correspondence to Author:
G. Ariharasivakumar

Professor

KMCH College of Pharmacy, Coimbatore- 641048, Tamil Nadu, India

E-mail: naviyamani@gmail.com

ABSTRACT: In the present study, high performance thin layer chromatographic method was developed and quantification of Diosgenin from *Albizia lebbeck*. TLC auminium precoated with silica gel were used as the stationary phase in a twin see through glass chamber saturated with n-butanol: glacial acetic acid: water (4:1:1) mobile phase, performed at room temperature (25ºC ± 2). The Rf value of diosgenin was found to be 0.8. Linearity was found to be in the concentration range 2 to 10 µg/spot. The linearity regression analysis of calibration plots showed good linear relationship between peak area and peak height (r^2= 0.996). The result showed that the chromatogram of Diosgenin was performed in 3D display of diosgenin and Overlay of diosgenin and ethanolic bark extract of *Albizia lebbeck*.

INTRODUCTION: India being blessed with a great biodiversity and abundance of flora has a rich heritage of traditional medicine constituting different components like *Ayurveda, Siddha* and *Unani*. Botanicals constitute the major part of these traditional medicines. The development of these traditional systems of medicines with the perspectives of safety, efficacy and quality will help not only to preserve the traditional heritage but also to rationalize the use of natural products in the healthcare [1]. Herbs and plants products have been used by man for combating diseases since times immemorial.

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.6(6).2610-15

Article can be accessed online on: www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.6(6).2610-15

The use of herbs for their therapeutic or medicinal value referred as Herbal medicine or Herbalism [2]. The Indian system of medicine is replete with medicinal plants that claim to promote learning, memory and intelligence. Extensive research is going on all around the world in different plants exploring the traditional system in treatment of cognitive disorders to have a relatively higher therapeutic window, lesser side effects and economical [3]. Wide varieties of plants have been investigated for their effect on cognitive functions of the brain and are termed as "Nootropic agents" [4].

Nootropic agents are any drug, supplement, nutraceutical, or functional food that is said to improve mental functions such as cognition, memory, intelligence, motivation, attention, and concentration. They are also known as memory enhancers. 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. TLC and HPTLC are methods commonly applied for the identification, assay and the testing of purity, stability, dissolution or content uniformity of raw materials (herbal and animal extracts, fermentation mixtures, drugs and excipients) and formulated products (pharmaceuticals, cosmetics, nutrients) [5]. Producing monomeric and polymeric phenols and polyphenols.

Albizia lebbeck Linn. **(**Mimosaceae) commonly called as *siris* is widely used in the treatment of many aliments. It has widely distributed all over India, mostly in Maharashtra, Punjab, Gujarat, Karnataka and Madhya pradesh. The bark of *Albizia lebbeck* contains tannins of condensed type, isomers of leucocyanidin, melacacidine, new leucoantho-cyanidin and lebbecacidin. It also contains triedelin and t_3 –sitosterol. The leaves of *Albizia lebbeck* used as an antiseptic and wound healing property. The bark of *Albizia lebbeck* used in the treatment of piles. It also possesses anti-spermatogenic and anti-inflammatory activity.

It is used in all types of poisoning, in skin eruptions, leprosy, leucoderma and wounds. Bark has acrid taste. The barks are used in toothache and diseases of the gum. It is recommended for bronchitis, leprosy, paralysis and helminth infections. The main constituents of the plant are saponins, macrocyclic alkaloids, anthraquinone glycosides, tannins, flavonoids and proteins. The saponin constituents of Albizzia so far described are echinocystic acid glycosides.

The albiziasaponins A, B, and C were isolated from the barks of A. lebbeck. Phytochemical investigations of Albizzia lebbeck pod showed that they contain 3', 5 Dihydroxy 4', 7 dimethoxy flavone, and N- Benzoyl L phenyl alaninol. The beans of the plant contain albigenic acid-a new triterpenoid sapogenin. Albizziahexoside a new hexaglycosylated saponin was isolated from leaves of *A. Lebbeck* [6]. N-demethyl budmunchiamines was isolated from *A. lebbeck* seeds [7]. This study was aimed to investigate HPTLC determination of bark extract but also compare with standard and in formulation.

MATERIAL AND METHODS:

Plant Collection and Authentication:

The Barks of *Albizzia lebbeck was* collected during the month of January 2013 from Nehru Nagar, Coimbatore, Tamil Nadu, India. The plant was identified and authenticated by A.K. Pradeep, Assistant Professor, Department of Botany, Calicut University Herbarium, Kerala, India.

Extraction of the Plant Material:

Plant material was washed with water, shade dried and the leaves was separated and powdered. The powdered material was defatted with petroleum ether and then successively extracted in Soxhlet apparatus with methanol (40-50 ºC). The extract was concentrated for further studies on water bath at 40 ºC.

High Performance Thin Layer Chromatography:

High performance thin layer chromatography (HPTLC) is a suitable quality assessment tool for the evaluation of herbal medicines and natural drug. Additionally, numerous samples can be run in a single analysis thereby it will reduce the analytical time. With HPTLC, the same analysis can be viewed at single and different wavelengths of light thereby providing a more complete profile of the plant and it is typically observed with more specific types of analysis.

The samples; diosgenin (2, 4, 6, 8 and 10 µL) and EEAL (4, 8 and 15µL) were spotted in the form of bands with a Camag microlitre syringe on pre-coated silica gel glass plate 60F-254 (10 × 10 cm with 0.2 mm thickness) using a Camag Linomat 5 applicator. The plates were pre-washed with methanol and activated at 60°C for 10 min prior to chromatography. The sample loaded plate was kept in TLC twin trough developing chamber after chamber saturation with respective mobile phase.

The optimized chamber saturation time for mobile phase was 10 min at room temperature (25 ± 2°C). Linear ascending development was carried out and the plate was developed in the respective mobile phase up to 7cm. The developed plate was then dried by hot air to evaporate solvents from the plate. The developed plate was observed under UV light at 254nm and 366nm and photo-documentation was performed. Bands were then

developed in iodine chamber and sprayed with specific reagent; anisaldehyde sulphuric acid. Finally, the plate was scanned 540nm using densitometer (Camag scanner 3) and operated by win CATS Planar Chromatography Manager [8].

RESULT AND DISCUSSION:

Chromatographic Screening Analysis:

HPTLC study was carried out for the quantification of diosgenin in extract. Visualization was performed as done in TLC (**Fig. 1**) and the selected mobile phase gave R_f values (0.85 ± 0.02) for diosgenin (**Table 1)**. After development, the plate was scanned in densitometer under 540 nm and the chromatogram obtained is depicted in **Fig. 2**. The calibration curve was prepared by plotting the concentration of diosgenin versus average area of the peak (**Table 2, Fig. 3**). The amount of diosgenin present in the extract was computed from calibration curve (**Table 3**).

TABLE 1: OBSERVATION OF TLC OF EEAL AND DIOSGENIN

Sl.No	Sample	No. of Spots	R_f Value
1	Diosgenin	1	0.8
2	EEAL	4	0.31, 0.74, 0.8, 0.9

TABLE 2: RESPONSES OBTAINED FOR DIOSGENIN IN PREPARATION OF CALIBRATION CURVE FOR HPTLC STUDIES

Track	R_f	Volume Applied (µl)	Amount Fraction (µg)	Area	Remark
1	0.88	2	2	5475.82	Diosgenin
2	0.86	4	4	7807.39	Diosgenin
3	0.85	6	6	9912.13	Diosgenin
4	0.85	8	8	10273.59	Diosgenin
5	0.83	10	10	9980.97	Diosgenin

TABLE 3: QUANTIFICATION OF DIOSGENIN IN EEAL SAMPLE BY HPTLC

Volume applied (µL)	Amount fraction (µg)	Area	Amount of diosgenin present (µg)	Diosgenin equivalent (DE mg/g)
8	800	10630.41	9.382	12.131 ± 0.404
15	1500	16036.27	18.803	

Note: Values are expressed as mean ± SD

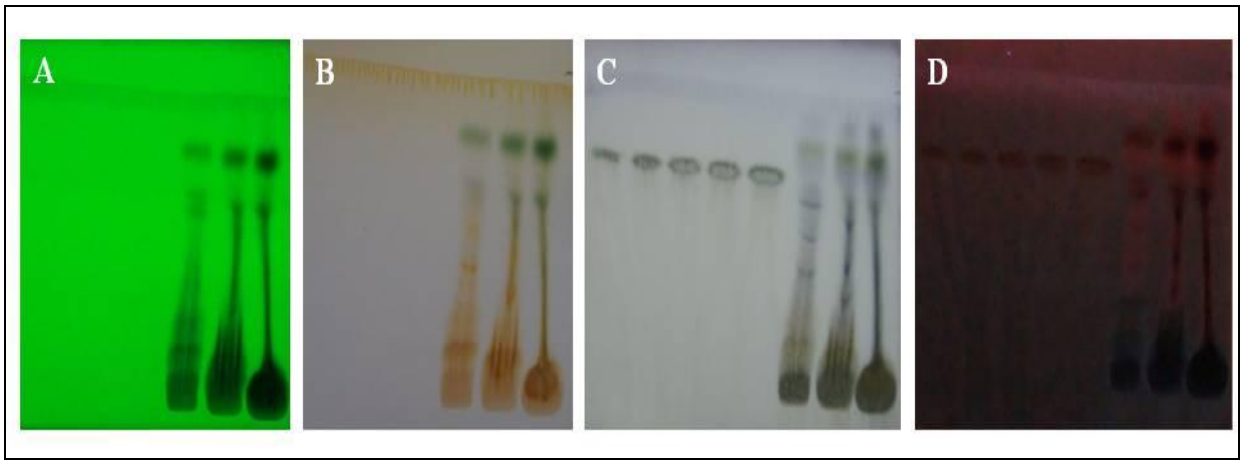


FIG.1: DETECTION OF BANDS

Bands were observed under UV at 254 nm (A), also developed in iodine chamber (B), and sprayed with anisaldehyde sulphuric acid reagent (C). UV detection at 366 nm (D) after drying in hot-air oven.

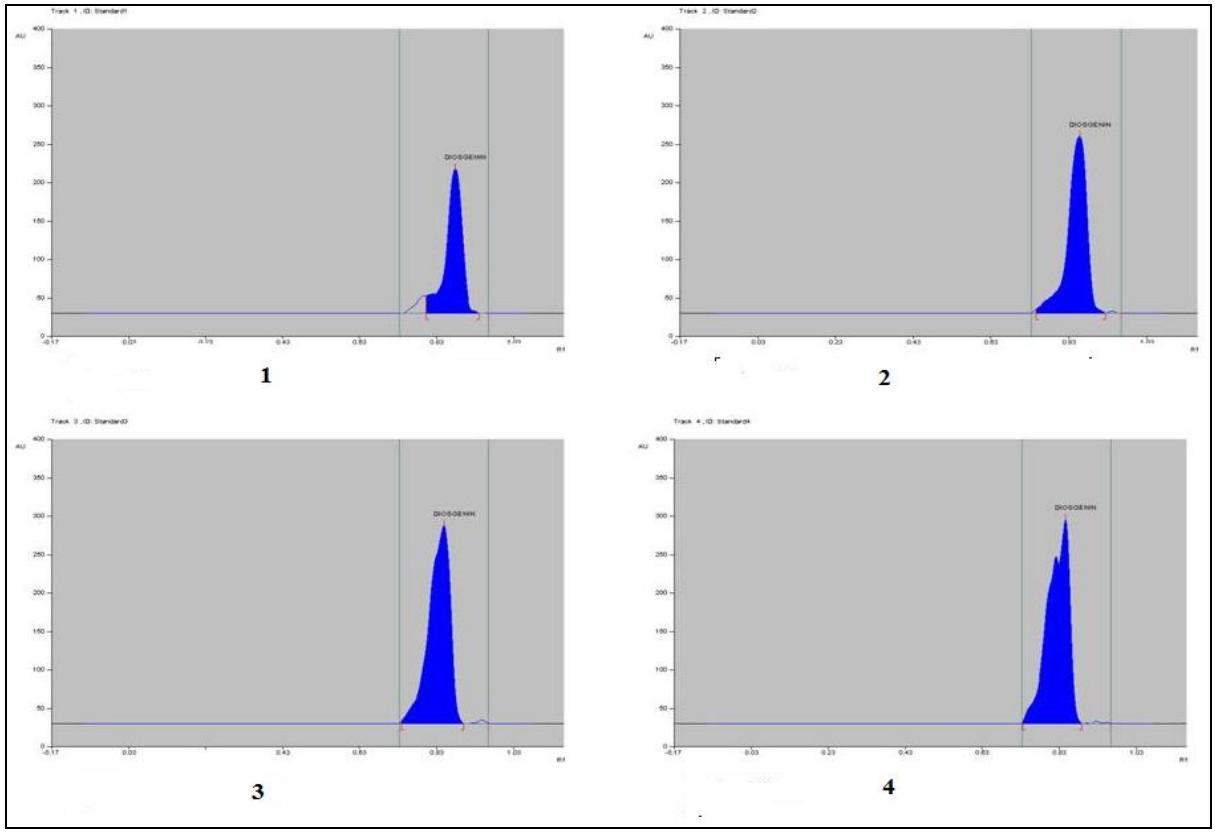
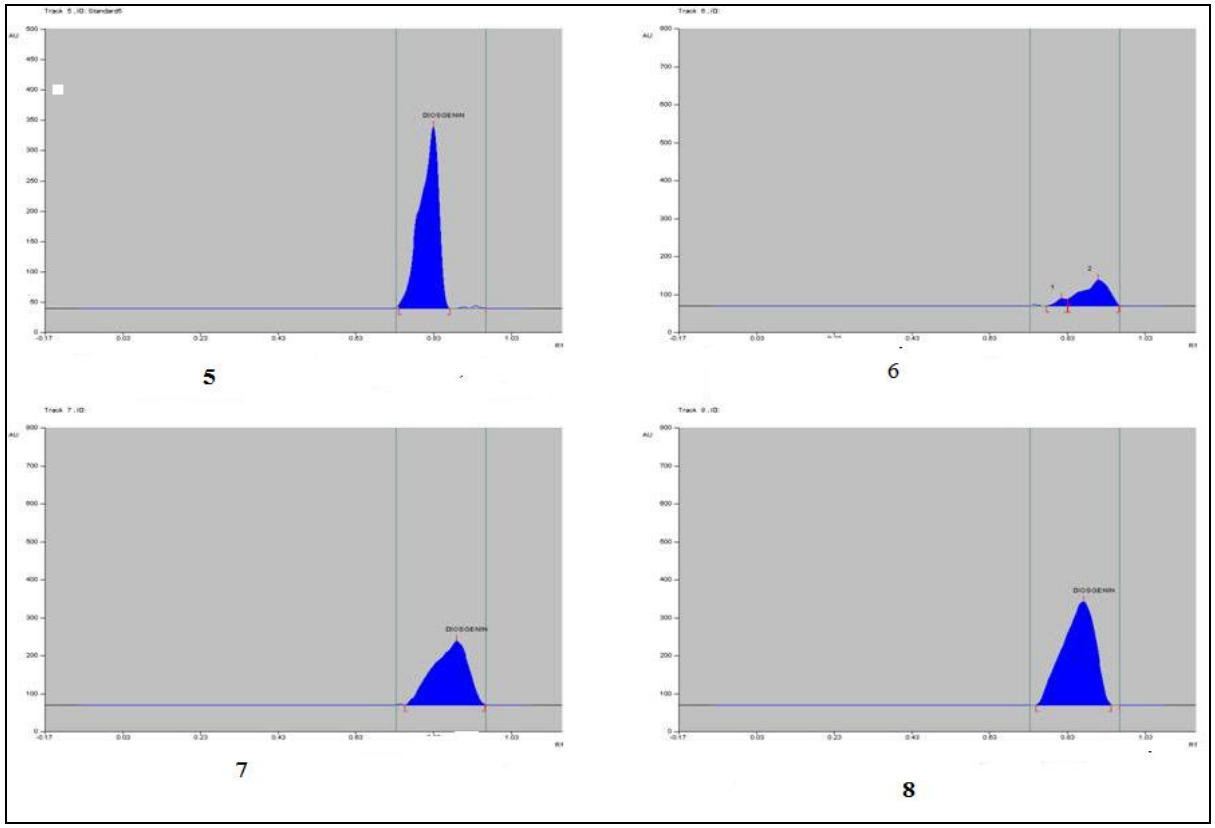


FIG.2: CHROMATOGRAM OF DIOSGENIN AND EEAL



1- Chromatogram of Diosgenin(2µl) , 2- Chromatogram of Diosgenin(4µl),
2- Chromatogram of Diosgenin(6µl), 4- Chromatogram of Diosgenin(8µl),
5- Chromatogram of Diosgenin(10µl), 6- Chromatogram of EE*AL*(4µl).
7- Chromatogram of EE*AL* (8µl), 8- Chromatogram of EE*AL*(15µl)

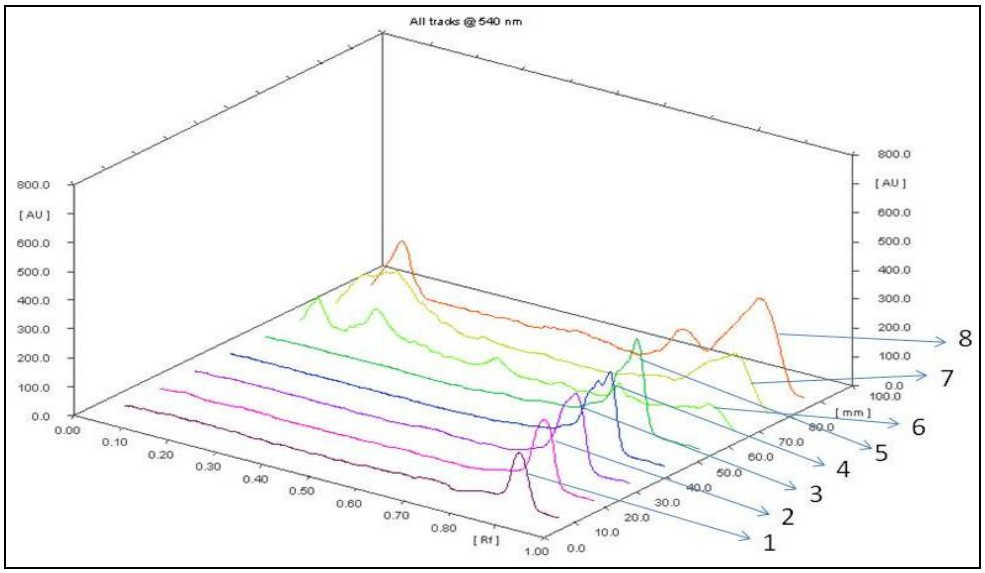


FIG. 3: 3D DISPLAY OF DIOSGENIN AND EEAL

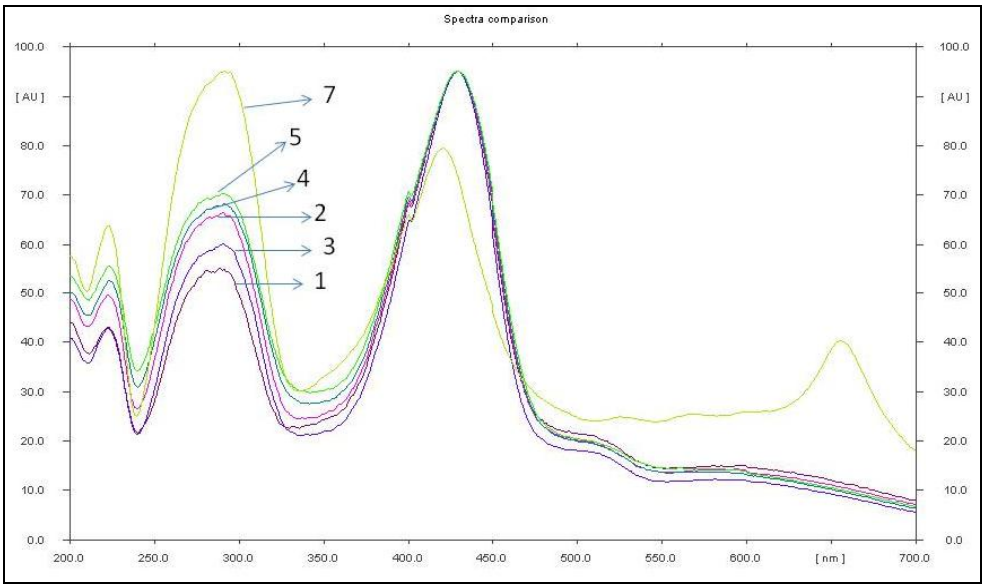


FIG 4: OVERLAY OF DIOSGENIN AND EEAL

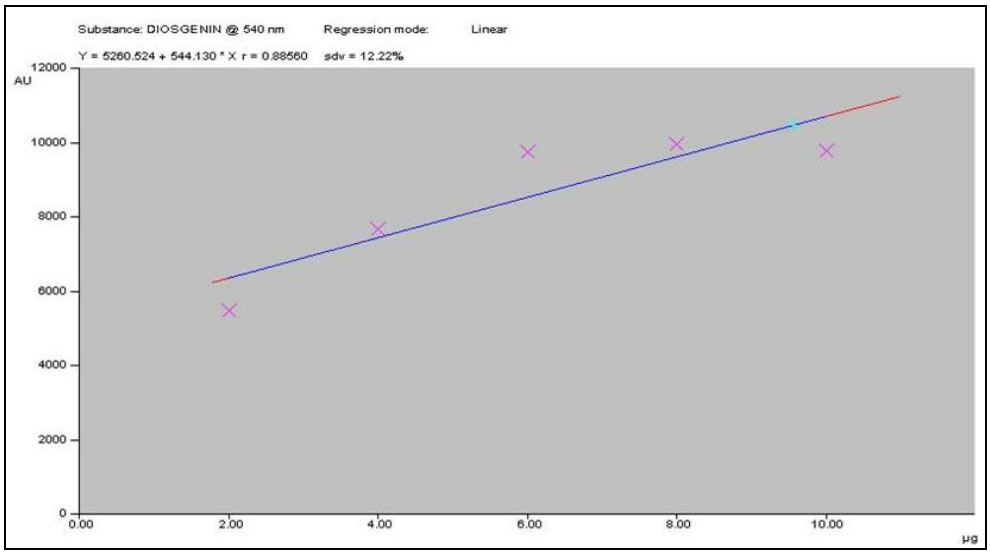


FIG. 5: CALIBRATION CURVE OF DIOSGENIN

In order to justify and quantify the presence of saponins, the extract was subjected to HPTLC screening against a marker compound, Diosgenin. Through literature survey, it was found that Diosgenin, a steroidal saponin has impressive pharmacological profile and been used for treatment of various type of disorders. It proved to have significant anticholinesterase activity [8] and hence diosgenin was used as the marker in HPTLC for the present study. From the results obtained from HPTLC study, it was found that EEAL contains 1.213% of diosgenin.

We express our gratitude to the Principal, Head Department of Pharmacy, M. Tamilarasan KMCH College of Pharmacy and Dr. S. Uma, SRM University for their support and continued encouragement

ACKNOWLEDGEMENT :

We express our gratitude to the Principal, Head Department of Pharmacy, M.Tamilarasan KMCH College of Pharmacy and Dr.S.Uma, SRM University for their support and continued encouragement.

REFERENCES:

1. Katekhaye, S.D, Bhutani K.K. Standardization of polyherbal Ayurvedic Formulation: *Ayaskrti*. Indian journal of traditional knowledge. 2011; 10(4): 589-593.
2. Faisal M, Singh P.P, Irchhaiya R. Review of *Albizzia lebbeck* a potent herbal drug. International research journal of pharmacy. 2012; 3 (5).
3. Soman I, Mengi S.A, Kasture, S.B. Effect of leaves of *Butea frondosa* on stress, anxiety and cognition in rats. Pharmacology, Biochemistry and Behaviour. 2004; 79: 11–16.
4. Shivakumar L, Shivaraj G.T, Rao N.V, Shalam R.V. Evaluation of nootropic activity of polyherbal formulation SR-105 in experimental animals. International research journal of pharmacy. 2011; 2 (4): 101-107.
5. Szepesi, G. Some aspect of the validation of planar chromatographic methods used in pharmaceutical analysis, I-General principles and practical approaches. J Planar Chromatogr, 1993; 6: 187-189.
6. Ueda M, Tokunaga T, Okazaki M, Sata N.U, Uda K, Yamamura S. Albizziahexoside; a hexaglycosylated saponin isolated from leaves of *Albizzia lebbeck*. Natural Product Research. 2003; 17: 29-35.
7. Mesra L.N, Dixit A.K, Wagner H. N-demethyl budmunchiamines from *Albizzia lebbeck* seeds. Phytochemistry. 1995; 39: 247–249.
8. Sobolewska S, Janeczko Z, Podolak P, Szerłomska A. Densitometric analysis of diosgenin in methanolic extracts of *Allium ursinum* collected at different times during plant development. Journal of Planar Chromatography - Modern TLC. 2009; 22: 305-307.

How to cite this article:
Kumar A, Ganesan and Subramanian K: High Performance Thin Layer Chromatography of Ethanolic Bark Extract from *Albizia Lebbeck* Linn. Int J Pharm Sci Res 2015; 6(6): 2610-15.doi: 10.13040/IJPSR.0975-8232.6(6).2610-15.