



Trade Science Inc.

June 2006

Volume 2 Issue 5-6

Analytical CHEMISTRY

An Indian Journal

Full Paper

ACAIJ, 2(5-6), 2006 [212-215]

HPTLC Finger Prints Of *Ephedra Gerardiana* Wall And *Sida Acuta* Burm



Rasheed-uz Zafar
Plant Tissue Culture Laboratory,
Department of Pharmacognosy and
Phytochemistry, Faculty of Pharmacy,
Jamia Hamdard,
New Delhi-110062 (INDIA)

Received: 2nd April, 2006

Accepted: 27th April, 2006

Web Publication Date : 10th June, 2006



Sayed Ahmad
Plant Tissue Culture Laboratory,
Department of Pharmacognosy and Phytochemistry,
Faculty of Pharmacy, Jamia Hamdard, New Delhi-110062 (INDIA)
E-mail: sahmad_jh@yahoo.co.in

ABSTRACT

High performance thin layer chromatography becomes a very important tool for identification and quantification of marker compounds as well as for checking purity and identity of the drugs. The HPTLC finger print profile of methanolic extracts of *Ephedra gerardiana* and *Sida acuta* was established for detection of marker compounds. The chromatogram was developed by using solvent system toluene: chloroform: ethanol (28.5: 57: 14.5) and scanned at 366 nm wavelength. Ephedrine (R_f 0.25) was found present in both the samples a new simple and rapid method for quantitative estimation of ephedrine in *Ephedra gerardiana* and *Sida acuta* was developed using standard ephedrine. The content of ephedrine was found 0.88 and 0.86 % w/w in *Ephedra gerardiana* stem and *Sida acuta* leaves respectively. © 2006 Trade Science Inc. - INDIA

KEYWORDS

Ephedra gerardiana;
Sida acuta;
Bariara;
Ephedrine;
HPTLC.

INTRODUCTION

Ephedra herba is a commonly used chinese crude drug for respiratory disorders. It consists of the dried aerial parts of *Ephedra* plant^[1]. The most common species of *Ephedra* used in India is *Ephedra gerardiana* Wall, for its usefulness in central nervous system (CNS), respiratory and cardiac disorders. The plant also has been used frequently by the practitioners of traditional system of medicine. The plant was found

to contain ephedrine as its main bioactive constituent^[2].

Sida acuta Burm. (Malvaceae), commonly known as Bariara, widely distributed throughout the tropical parts of India. The plant is under shrub or shrub, which has importance for its medicinal value and also as a substitute of jute for yielding fiber. The leaves of the plant are considered to possess demulcent, diuretic properties and are used in rheumatism, testicular swelling and also in elephantiasis. The aerial

part of the plant was found to contain significant amount of alkaloids and the major portion of which is ephedrine^[3]. Ephedrine is a phenyl alkyl amine alkaloid found to possess CNS stimulant^[4], anti-asthmatic, anti-inflammatory, anti-histaminic and adrenergic properties. Ephedrine has advantage over adrenaline that it can be given orally and its action is prolonged^[5], now days it is also used as a common ergogenic drug^[6,7,8].

In view of the importance of *Ephedra gerardiana* and *Sida acuta* in traditional system of medicine and use of ephedrine for various disorders, it was thought worthwhile to develop a simple, rapid and reproducible high performance thin layer chromatography (HPTLC) finger print for these drugs. In the present investigation an attempt has therefore been made to establish the HPTLC finger print for detection of marker compounds in both the plants and also to develop simple, rapid, reproducible and sensitive method for quantitative estimation of ephedrine in crude drugs using HPTLC system.

EXPERIMENTAL

Plant material

Sida acuta plant leaves were collected from herbal garden of Hamdard University, New Delhi, during the month of November and *Ephedra gerardiana* stem was purchased from the local drug market (Kharibawli, Delhi). Both the drugs were identified by the Department of Botany and voucher specimens were deposited at the herbarium of University. Drugs were dried at room temperature bellow the fan for seven days with continuous turning to assure uniform drying. The dried leaves of *Sida acuta* and stem of *Ephedra gerardiana* were powdered and sieved through # 100. The sieved powders were used for further work.

Preparation of samples

Dried and powdered leaves of *S. acuta* and stem

of *E. gerardiana* (5 gm each) were macerated in methanol (25 ml each) with stirring for 24 hrs in two separate 250 ml conical flasks. The content of the flasks were filtered and washed three times with methanol (5 ml each) to assure complete extraction. Filtrates were then dried under reduced pressure and both the residues left were re-dissolved in five-ml of methanol using two separate standard flasks, which was used for application on TLC plate.

Standard ephedrine

Ephedrine was isolated from *E. gerardiana* stem using the method described by Peach and Tracy^[9]. The purity of isolated ephedrine was checked by TLC of its hydrochlorides in three different solvents^[10], mp. (42 °C) and also by matching UV spectra (λ_{max} value of 0.05 % w/v solution in 0.1N HCl was found 259 nm), which was found exactly similar to that reported in Pharmacopoeia^[11].

HPTLC finger print

Two micro-liter each of methanolic extracts of *Ephedra gerardiana* stem and *Sida acuta* leaves were applied in duplicate on TLC plate. The chromatogram was developed and scanned using Camag densitometric scanner-3 equipped with winCats software. The HPTLC finger print of both the drugs were established (Figure 1), number of spots and their Rf values of different tracks are recorded in TABLE 1. Quantitative estimation of ephedrine: Accurately weighed 10 mg of standard ephedrine (isolated and characterized) was dissolved in five ml of methanol to get two mg/ml solution, which was used for application. Five micro liter of standard ephedrine solution and two micro liters each of samples were applied in duplicate on TLC plate. The content of ephedrine was estimated in crude drugs from the standard plot (concentration of standard ephedrine vs. mean peak height of standard), using single level calibration. The standard deviation of calibration curve and percentage coefficient of variance (% CV)

TABLE 1: HPTLC finger print of methanolic extracts of *Ephedra gerardiana* stem and *Sida acuta* leaves

Sr.	Track no.	Plant/Drug	No. of spots	Rf values
1	1-2	<i>Ephedra gerardiana</i>	09	0.06, 0.21, 0.25, 0.32, 0.48, 0.52, 0.70, 0.74, 0.89
2	3-4	<i>Sida acuta</i>	10	0.21, 0.25, 0.32, 0.37, 0.62, 0.70, 0.79, 0.82, 0.91, 0.95

Full Paper

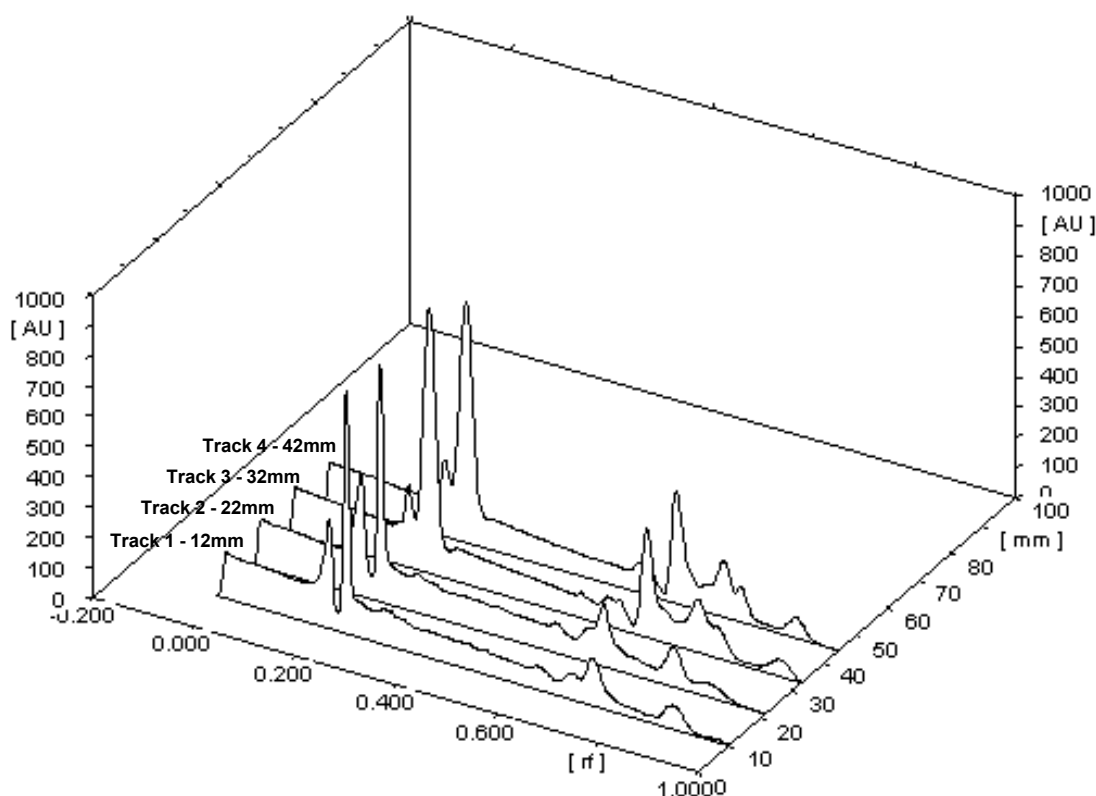


Figure 1: Chromatogram showing peaks of different spots of methanolic extracts of *Ephedra gerardiana* stem (track 1-2) and *Sida acuta* leaves (track 3-4).

of samples were also calculated using winCats software to find the reproducibility of the method.

Chromatographic conditions

Instrument

Camag HPTLC system, consisting of Linomat V spotting device and Scanner III with winCats 4 software.

Stationary phase

TLC aluminum sheets silica gel 60 F₂₅₄ pre coated layer (10 cm x 10 cm), thickness 0.2 mm.; Mobile Phase: toluene: chloroform: ethanol (28.5: 57: 14.5)^[12], Solvent front: 68 mm.; Scanning wavelength – 366 nm, Measurement mode – fluorescence, Lamp – deuterium & tungsten (D₂ & W).

RESULTS AND DISCUSSION

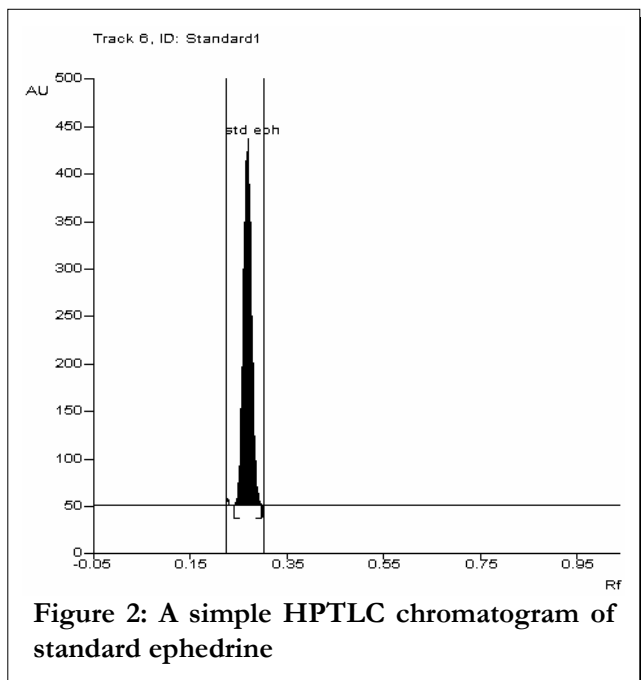
From the qualitative work done on the methanolic extracts of *Ephedra gerardiana* stem and *Sida acuta* leaves it can be concluded that significant

differences exist between the chemical constituents of both drugs (TABLE 1). This has evidenced from the HPTLC finger print (Figure 1) that spots of compounds having Rf value 0.21, 0.25 (ephedrine), 0.32, 0.70 and 0.82 are common in both the drugs, while spot of compounds having Rf value 0.37, 0.62, 0.79, 0.91 and 0.95 are only present in *Sida acuta* leaves. The spot of compounds having Rf value 0.06, 0.48, 0.52, 0.57, 0.74 and 0.89 are only characteristic to *Ephedra gerardiana* stem.

Chemical tests^[11] and TLC^[12] reveal the presence of ephedrine a bioactive anti-asthmatic compound^[5] in the methanolic extracts of *Ephedra gerardiana* stem and *Sida acuta* leaves. Ephedrine was estimated quantitatively in both the drug samples using Camag HPTLC system and it was found that *Ephedra gerardiana* stem and *Sida acuta* leaves do not differ much in ephedrine content. Three-dimensional view of the drug samples and standard are shown in figure 2, and the results of quantitative estimation of ephedrine are recorded in TABLE 2.

TABLE 2: Quantitative estimation of ephedrine in *Ephedra gerardiana* stem and *Sida acuta* leaves

Sr.	Drug	Amount of ephedrine (% w/w)	% CV
1	<i>Ephedra gerardiana</i>	0.88	4.88
2	<i>Sida acuta</i>	0.86	8.57

**Figure 2: A simple HPTLC chromatogram of standard ephedrine**

The standard deviation of the single level calibration curve was found to be 10.34, which shows reproducibility of the method and low percentage coefficient of variance (% CV) of drug samples reveals the reproducibility of the results.

The results obtained in the present investigation are significant from the point of view that the HPTLC finger print profile established for *Ephedra gerardiana* stem and *Sida acuta* leaves can be used for checking purity, identity, substitutes and adulterants of these drugs. The simple, rapid, reproducible and sensitive method developed for quantitative estimation of ephedrine can be used for quality control of crude drugs as well as for ephedrine containing formulations. It is also concluded from the present investigation that the *Sida acuta* leaves can be used as an alternative source for the production of ephedrine.

REFERENCES

- [1] Anonymous; 'The Wealth of India Raw Materials', Publication & Information Directorate, CSIR, New Delhi, **III**, 82 (1976).
- [2] R.N.Chopra, S.L.Nayar, I.C.Chopra; 'Glossary of Indian Medicinal Plants', Publication and Information Directorate, CSIR, New Delhi., 108 (1956).
- [3] Anonymous; 'The Wealth of India Raw Materials', Publication and Information Directorate, CSIR, New Delhi., **IX**, 322 (1976).
- [4] R.P.Rastogi, B.S.P.Mehrotra; 'Compendium of Indian Medicinal Plants', Publication and Information Directorate, CSIR, New Delhi., 586 (1993).
- [5] W.C.Evans; 'Trease and Evans Pharmacognosy', Harcourt Brace and Company Asia PTL Ltd., 365 (1996).
- [6] M.E.Powers; J.Athl.Train., **36(4)**, 420-424 (2001).
- [7] K.M.Kuczowski; Acta Obstet.Gynecol.Scand., **85(2)**, 247-248 (2006).
- [8] R.Calfee, P.Fadale; Pediatrics, **117(3)**, 577-589 (2006).
- [9] K.Peach, M.V.Tracy; 'Modern Methods of Plant Analysis', Springer-Verlag, UK., **IV**, 418 (1955).
- [10] P.D.Sethi; 'Quantitative Analysis of Drugs in Pharmaceutical Formulations', CBS Publisher, New Delhi., 187,188,197,199,352,354,368 (1997).
- [11] Anonymous; 'Pharmacopoeia of India', 3rd Ed., **II**, Publication and Information Directorate, CSIR, New Delhi, 187 (1985).
- [12] H.Wagner, S.Bladt; 'Plant Drug Analysis', Springer-Verlag, Berlin, Hidelberg, UK, 46 (1996).