THIN LAYER CHROMATOGRAPHY AS A TOOL FOR QUALITY CONTROL OF PUNARNAVA

Aradhya C. Vijayakrishna1, Shreedhara C. S., Lattha K. P., Adavirao V. Belvotagi
1Research Scholar, Department of Pharmaceutical Chemistry, National College of Pharmacy, Shimoga, Karnataka, India
2Prof and Head, Department of Pharmacognosy, Manipal College of Pharmaceutical Sciences, Manipal University, Manipal, Karnataka, India
3Assistant Professor, Department of Chemistry, Sahyadri Science College, Shimoga, Karnataka, India
4Independent researcher

DOI: 10.7897/2277-4572.034177
Published by Moksha Publishing House. Website www.mokshaph.com
All rights reserved.

Received on: 05/07/14 Revised on: 01/08/14 Accepted on: 12/08/14

ABSTRACT
Quality Control of Punarnava is highly essential for obvious reasons. Available methods fail to ensure quality of Punarnava in a rapid, simple and economical way. A TLC method incorporating the above objectives has been developed using Chloroform: Ethyl acetate: methyl alcohol (4:4:2) / Chloroform: Methanol (85:15) as solvent system and as Alcoholic sulphuric acid (10 %) visualizing agent. This method seems to be reasonably good and refinements to fine tune the results are underway.

Keywords: Phytoconstituents, Boerhaavia diffusa, Trianthema portulacastrum TLC, Punarnavine.

INTRODUCTION
Ayurveda is a native Indian healthcare system which is currently used by millions of people in India and across the world for their day-to-day healthcare needs. Plants have great potential uses especially, as traditional medicine and Pharmacopoeial drug. Boerhaavia diffusa Linn (BD) or Punarnava has been used in the indigenous medicine from time immemorial. The Ayurvedic authorities recognized two varieties of this plant, the one with white flowers called ‘swetha punarnava’, and the other with red flowers, the ‘rakt punarnava’. The plant grows all over India as a common creeping weed and is especially abundant during rain. Boerhaavia diffusa Linn (Nyctaginaceae) is an herbaceous plant, occurs abundantly as a weed throughout India. It is a creeping and spreading perennial herb, with a stout root-stock and many erect or spreading branches and also cultivated in fields, spreading widely distributed in the tropical and subtropical regions in the world. Market samples of Boerhaavia diffusa are often adulterated with Trianthema portulacastrum Linn (TP) may be because of their common name “Punarnava”. These two plants are the sources of two different Ayurvedic drugs viz., Punarnava (Raktpunarnava) and Varshabhu (Swetha Punarnava), the roots of which are claimed to possess similar therapeutic effects. The two plants differ widely in their stomatal indices and palisade ratios; Trianthema portulacastrum possessing higher values. The Boerhaavia diffusa could be substituted with the qualitatively inferior, abundantly available and commercially attractive plant material as adulterant i.e. Trianthema portulacastrum. Trianthema portulacastrum Linn (TP) belongs to family Aizoaceae1,4.

MATERIAL AND METHODS
Plant collection and Authentication
The whole plant materials were collected from different natural habitats from Javalli village, Shimoga district of Karnataka, India during August 2013, were washed thoroughly with purified water, chopped, dried under shade, powdered and stored in polythene bags at 4°C till further analysis. The plant was authenticated by Dr. Krishna Swamy, Associate professor, Department of Botany, Sahyadri Science College, Shimoga, Karnataka, India.

Chemicals used
Ethanol, methanol, ethyl acetate, chloroform, acetic acid, iodine, ceric ammonium sulphate, sulphuric acid, etc were of AR Grade and purchased from E Merck and Hi-Ar Grade and purchased from E Merck and Hi-Media, Mumbai, India. Marker compounds were procured from Natural Remedies, Bangalore, Karnataka, India. Double distilled water was used whenever necessary. Pre-coated silica gel on aluminum analytical TLC Plates Silica gel 60 F254 purchased from Merck were used. Commercial crude drugs, roots of Boerhaavia diffusa and Trianthema portulacastrum were purchased from Amruth Kesari Depot, Bangalore, Karnataka, India.

Phytochemical screening
The freshly prepared crude extracts of Boerhaavia diffusa is qualitatively tested for the presence of Alkaloids (Hager's test), Flavonoids (Modified Ammonia test), Steroids (Salkowski test), Terpenoids (Modified Salkowski test), Reducing sugars (Fehling's test), Saponins (Frothing test), Tannins (FeCl3 test), and Anthraquinones (Chloroform layer test). Boerhaavia diffusa has been widely studied for its chemical constituents and therapeutic activities. The roots are the source of a novel class of iso flavonoids known as rotenoids, flavonoids, flavonoids, glycosides, xanthenes, purine nucleoside, lignans and steroids. Punarnavine (C17H22N2O m. p. 236–237°C) is an alkaloid present in the plant Boerhaavia diffusa. Various animal studies and trials have confirmed the presence of activities, for example, immunomodulation, hepatoprotective, antihypertensive, Anti fibrinolytic, anticancer activity, anti-diabetic activity, anti-inflammatory and diuretic. Trianthema portulacastrum is a prostrate, glabrous, succulent annual found almost throughout India as a weed in cultivated and wastelands. The plant is bitter, alecteric, analgesic, stomachic, laxative and serves as alterative cures for bronchitis, heart disease, blood anemia,
inflammation and piles. The root applied to the eye cures corneal ulcers, itching, dimness of sight and night blindness\(^5\) -\(^7\). The detailed analytical profiles of the extracts/fractions of *Trianthema portulacastrum* and *Boerhaavia diffusa* have been carried out by different analytical techniques as follows: GC (alcohol content, AAS (metal content) HPLC\(^8\), (Pesticide residue, Eupalitin galactoside); HPTLC\(^9\) (markers like Boeravonine B, Eupalitin galactoside, β sitosterol); MAE-HPTLC\(^9\) (Boeraveravonine B and E); TLC\(^11\); UV, IR, HPLC, MS and \(^1\)HNMR\(^13\); quantitative TLC and HPTLC (β sitosterol)\(^13\). TLC, HPTLC\(^14\) (Flavonoids). But none of these analytical techniques consider the economy of the recourses and labor in order to achieve these objectives; we have tried to develop a new TLC method which can be considered as reasonably good.  

The method being reported herewith would be simple, rapid, and inexpensive method for identifying and comparing the major constituents of *Boerhaavia diffusa* and *Trianthema portulacastrum*. This method in contrast to above ones provides a common visualizing agent for majority of the components and the TLC system used provide the best resolution possible for the major constituents of *Trianthema portulacastrum* and *Boerhaavia diffusa*. This would further help in quantitative estimation of individual components there by making the comparison between *Trianthema portulacastrum* and *Boerhaavia diffusa* more accurate.

### Preparation of Extracts

The whole plant of *Boerhaavia diffusa* and *Trianthema portulacastrum* was shade dried and the dried whole plants / commercial roots were powdered to get coarse powder. The coarse powder was subjected to extraction using Methanol by cold maceration method. The solvent was removed by distillation under reduced pressure, which produced a greenish sticky residue (yield 10 %w/w). The concentrated crude extract were stored in pre weighed air tight contraries and used for the further study.

![Figure 1: Thin Layer chromatograms of methanolic extracts of *B. diffusa* and *T. portulacastrum* as resolved in various solvent systems and visualizing agents](image)

**Figure 1:** Thin Layer chromatograms of methanolic extracts of *B. diffusa* and *T. portulacastrum* as resolved in various solvent systems and visualizing agents

Punarnavine II and Punarnavoside
RESULTS

TLCs were performed based on the literature available in monographs and other publications. The objective of this study was to find a simple, rapid, inexpensive method for identifying the major constituents of *Boerhaavia diffusa*, so that it can be used as a simple quality control tool. Because, *Trianthema portulacastrum* is a known adulterant of *Boerhaavia diffusa*, if the method is able to tell whether *Trianthema portulacastrum* is present as an adulterant in *Boerhaavia diffusa*, it would be an added advantage. With the aim of achieving the dual objective, the study was planned. Initial studies revealed some facts as shown below:

- A single solvent system is not enough to resolve all the ingredients.
- A single visualizing system also is of no use in identifying them.

TLC profiles of *Boerhaavia diffusa* and *Trianthema portulacastrum* indicate almost similar if not identical in both polar and non-polar zones. If at all a simple method for qualitative and quantitative analysis is to be developed under these circumstances, modification of solvent systems, evaluation of number of non specific visualizing agents and isolation of active constituents (initially) is essential. Equipped with the above info, fresh studies involving modified solvent systems, non-specific visualizing agents and isolated active ingredients (Punarnavine I and II and Punarnavoside) and markers compounds (Eupalitin, Boerhaavinone B and Eupalitin 3-O-galactoside) were carried out. The results of these studies (Figure 1-3) are as shown below and from these following inferences could be made: Simple solvent systems like Chloroform: Methanol, Chloroform: Acetone, Chloroform: Ethyl Acetate: Acetic acid, MeOH: Chloroform: Acetic acid: Benzene with moderate polarity could be able to resolve all the ingredients very well. Of the non-specific visualizing agents {UV-365, Iodine Vapor, Alcoholic H$_2$SO$_4$ (10 %)} and specific visualizing agent for alkaloids i.e., Ceric Ammonium Sulphate (10 % Aq.) were found to be satisfactory. Especially for visualizing Punarnavine I and II and Punarnavoside and the three marker compounds, Alcoholic H$_2$SO$_4$ (10 %) would be the best. With respect to the choice of solvent system for the qualitative and quantitative evaluation of *Boerhaavia diffusa*, Chloroform: Methanol (85:15) or Chloroform: Ethyl Acetate: MeOH (4:4:2) could be the best ones as per the studies conducted so far. Qualitative differentiation of *Boerhaavia diffusa* from *Trianthema portulacastrum* is possible based on the TLC profiles of the two as evident in many plates in Figure 3. The TLC analysis clearly justifies the use of *Trianthema portulacastrum* as a substitute for *Boerhaavia diffusa* as it is found to match with *Trianthema*.
portulacastrum both in terms of number of components and their relative concentrations. However, the TLC profiles of the two are not identical.

REFERENCES

Source of support: Nil, Conflict of interest: None Declared