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Research Article

THIN LAYER CHROMATOGRAPHY AS A TOOL FOR QUALITY CONTROL OF PUNARNAVA

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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 portulucastrum* 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 portulucastrum* Linn (TP) may be because of their common name "Punarnava". These two plants are the sources of two different Ayurvedic drugs viz., Punarnava (Raktapunarnava) and Varshabhu (Swetha Punarnava), the roots of which are claimed to be possessing similar therapeutic effects. The two plants differ widely in their stomatal indices and palisade ratios; *Trianthema portulucastrum* 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 portulucastrum*. *Trianthema portulacastrum* Linn (TP) belongs to family Aizoaceae[1-4].

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, cerric ammonium sulphate, sulphuric acid, etc were of 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 F 254 purchased from Merck were used. Commercial crude drugs, roots of *Boerhaavia diffusa* and *Trianthema portulucastrum* 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 ($FeCl_3$ 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 isoflavonoids known as rotenoids, flavonoids, flavonoids, glycosides, xanthenes, purine nucleoside, lignans and steroids. Punarnavine ($C_{17}H_{22}N_2O$ 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 portulucastrum* is a prostrate, glabrous, succulent annual found almost throughout India as a weed in cultivated and wastelands. The plant is bitter, alexiteric, 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 portulucastrum* and *Boerhaavia diffusa* have been carried out by different analytical techniques as follows: GC (alcohol content, AAS (metal content) HPLC[8], (Pesticide residue, Eupalitin galoctoside); HPTLC[9] (markers like Boeravinone B, Eupalitin galoctoside, β sitosterol); MAE-HPTLC[10] (Boeraveravinone B and E); TLC[11]; UV, IR, HPLC, MS and ^{1}HNMR[12]; 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 portulucastrum*. This method in contrast to above ones provides a common visualizing agent for majority of the components and the TLC system used provides the best resolution possible for the major constituents of *Trianthema portulucastrum* and *Boerhaavia diffusa*. This would further help in quantitative estimation of individual components there by making the comparison between *Trianthema portulucastrum* and *Boerhaavia diffusa* more accurate.

Preparation of Extracts

The whole plant of *Boerhaavia diffusa* and *Trianthema portulucastrum* 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.

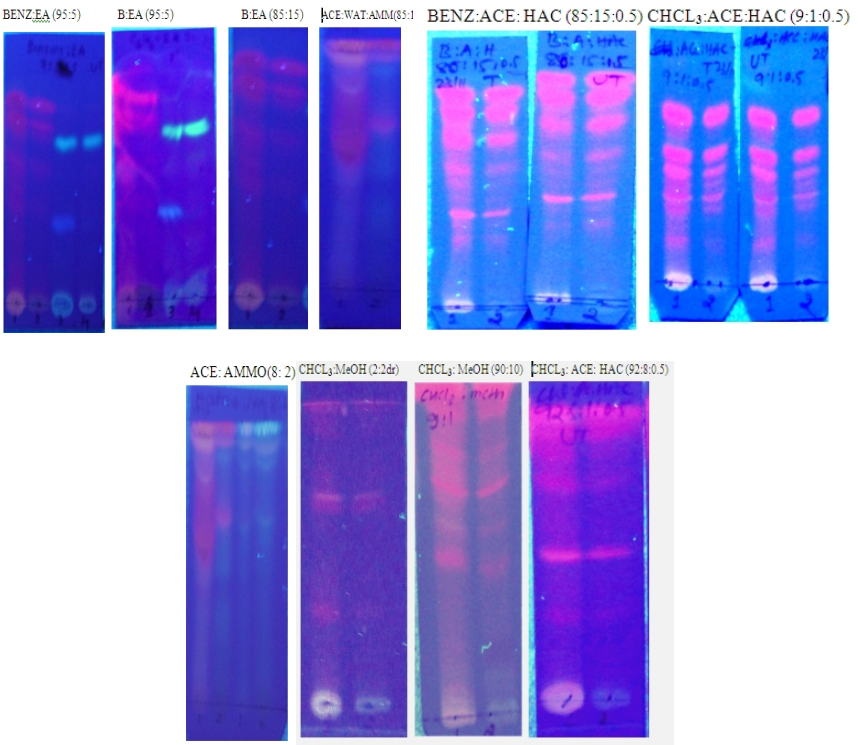

Figure 1: Thin Layer chromatograms of methanolic extracts of *B.diffusa* and *T.portulucastrum* as resolved in various solvent systems and visualizing agents

Punarnavine II and Punarnavoside

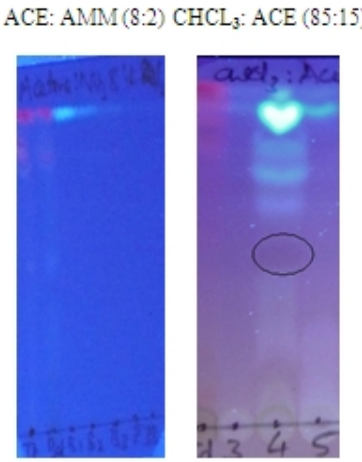

Figure 2: Comparative Thin Layer Chromatograms of the isolates or the fractions rich in major active ingredients of *B. diffusa* with that of methanolic extracts of *B. diffusa* and *T. portulucastrum*
Punarnavine I and II, Punarnavoside, Eupalitin, Boerhaavinone B and Eupalitin 3-O- galoctoside

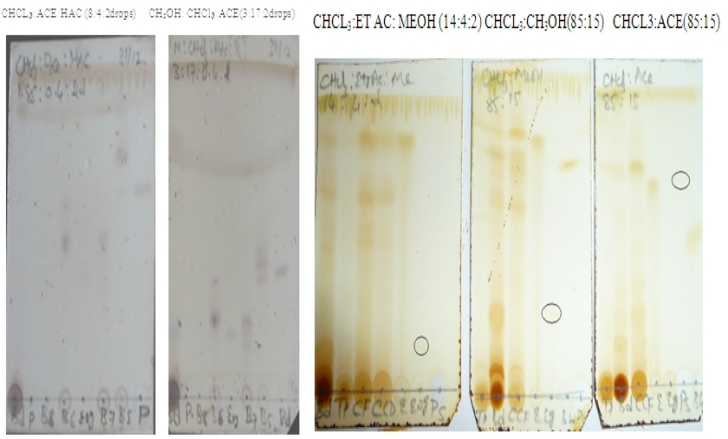

Figure 3: Comparative Thin Layer Chromatograms of the isolates or the fractions rich in major active ingredients of *B. diffusa* with that of methanolic extracts of *B. diffusa* and *T. portulucastrum*

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 portulucastrum* is a known adulterant of *Boerhaavia diffusa*, if the method is able to tell whether *Trianthema portulucastrum* 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 planed. 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 portulucastrum* 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_2SO_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_2SO_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 portulucastrum* 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 portulucastrum* as a substitute for *Boerhaavia diffusa* as it is found to match with *Trianthema*

portulucastrum both in terms of number of components and their relative concentrations. However, the TLC profiles of the two are not identical.

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