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HPTLC Densitogram Profile of Aqueous Distillate *(Arka)* from Roots of *Boerhaavia diffusa*

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Authors' contributions

This work was carried out in collaboration between all authors. Authors VKL and Pooja designed the study, wrote the protocol, and wrote the first draft of the manuscript. Author AV managed the literature searches, analyses of the study performed the spectroscopy analysis and author Pooja managed the experimental process and identified the species of plant. All authors read and approved the final manuscript.

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ABSTRACT

The aqueous distillate (arka) of *Boerhaavia diffusa* was prepared and investigated qualitatively for TLC. The following work constitutes the enrichment of constituents present in the aqueous distillate (arka) by partition technique using various solvents such as toluene and ethyl acetate in the ratio of (7:3), toluene:acetone:acetic acid (8.5:1:0.5) chloroform:methanol (9:1) Enrichment was determined by Thin Layer Chromatography and HPTLC. Hence it was concluded that aqueous distillate showed the better result for TLC in different solvent systems tried, but best solvent system selected for TLC was toluene: acetone: acetic acid in the ratio of (8: 0.5:1.5) using 5% methanol-sulphuric acid solution as detecting reagent.

Keywords: TLC; toluene; acetone; acetic acid; B. diffusa.

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1. INTRODUCTION

Boerhaavia diffusa Linn. (syn. *B. repens* Linn. var. *procumbens* Hook.f.) belongs to the family Nyctaginaceae and is known as Punarnava in Sanskrit and Hogweed in English [1].

Boerhaavia diffusa is a perennial creeping weed, prostrate or ascending herb, up to 1 m long or more having spreading branches. The plant grows profusely in the rainy season, and mature seeds are formed in October-November. Due to sticky nature it gets stuck on the clothes of human beings and on the legs of animals, which helps to the dispersion of plant from one place to another [2].

The plant has drawn lot of attention due to its uses in Indian Traditional Medicine. The various parts of the plant are used in the treatment of cancer, jaundice, dyspepsia, inflammation, ophthalmic, enlargement of spleen, abdominal pain and as an anti-stress agent [3].

Boerhaavia diffusa plant contains a large number of compounds such as flavonoids (retinoid) (coumarins), alkaloids (quinines) terpenes (triterpenoids), steroids and proteins [4,5].

The roots are reputed to be diuretic and laxative and are given for the treatment of anasarca, ascites and jaundice [6]. The root juice is used to cure asthma, urinary disorders, leukorrhea, rheumatism, and encephalitis [7,8]. The worldwide use of *B. diffusa* roots to treat liver disorders was validated when researchers demonstrated, in 1980 and 1991 that its root extract had anti-hepatotoxic properties [8].

Thus after a wide survey of literatures and ancient Ayurvedic books about roots of *Boerhaavia diffusa* here the aim is to find out the chemical constituents present in aqueous distillate by using HPTLC method, Rf values were compared, by identifying TLC of plant *Boerhaavia diffusa* in Ayurvedic Pharmacopoeia and other research articles.

2. MATERIALS AND METHODS

2.1 Equipment for Densitograms

The HPTLC instrument consisted of a CAMAG (Muttenz, Switzerland) Linomat V sample applicator with a 100-µL applicator syringe (Hamilton, Bonadauz, Switzerland). Chromatography was performed on 10 cm × 10 cm aluminium TLC plates precoated with silica gel 60-F254 (E. Merck, Darmstadt, Germany; supplied by Anchrom Technologists, Mumbai, India). A CAMAG TLC scanner 4 was used for densitometric scanning of the chromatogram.

2.2 Plant Material

The plant drug (roots) was collected from local land of Lucknow Uttar Pradesh India. The plant was collected in the month of December, 2013. The shade dried plant material was authenticated in National Botanical Research Institute NBRI Lucknow India. Voucher specification no is (NBRI-SOP-202) and grounded to coarse powder for distillation with water. HPLC analytical grade solvents and reagents were used for TLC purpose. Silica gel G plates 0.2 mm thickness alumina base from E. Merck Ltd, Mumbai, India, were used.

2.3 Preparation of Aqueous Distillate (*Arka)*

Arka is a liquid preparation obtained by distillation of certain liquids or drugs soaked in water using the *Arkayantra* or any convenient modern distillation apparatus [9]. The (1:16) (62.63 gm. /1000 ml) ratio sample of powdered *Boerhaavia diffusa* roots was extracted in 200 ml distilled water was added to the roots for soaking and kept over-night for 12 hrs. This makes the roots soft and when boiled releases the essential volatile principles easily. The following morning it is poured into the *Arkayantra* (distillation assembly) and the remaining 800 ml distilled water was added to make up the volume and boiled. The vapour was condensed and collected in a receiver. In the beginning, the vapour consists of only steam and may not contain the essential principles of the extract. It should therefore be discarded. The last portion also may not contain therapeutically essential substance and was discarded. Ayurvedic Formulary of India Part I (2001) pg. 27-28 [9]. The aliquots collected in between contain the active ingredients and may be mixed together to ensure uniformity of the *Arka*. The volume of 5 ml from it, used for TLC in three ratio (5 µl, 10 µl, 15 µl) using a micro syringe [9].

2.4 Sample Application

A volume of (5 µl, 10 µl, and 15 µl) was applied in the form of circular spot by micro syringe on

the silica gel 60 F254, E merk TLC plate of 0.2 mm thickness [10].

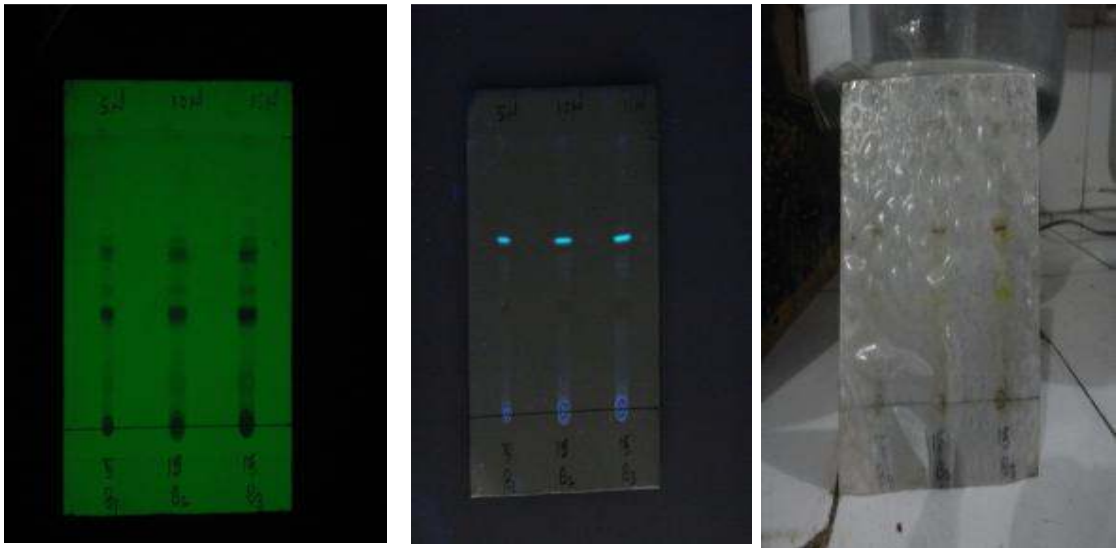
2.5 Development of Plate

The plate was developed in a solvent system toluene: acetone: acetic acid (8: 0.5:1.5) and spray with methanol-sulphuric acid reagent [11].

3. RESULTS AND DISCUSSION

The result is only for presence of compounds in this solvent system at concentrations of aq distillate of *Boerhaavia diffusa* (5 µl, 10 µl, and 15 µl) [12]. The components are found first time with this solvent system [13]. Aqueous distillate of root of plant *Boerhaavia diffusa* was prepared by simple distillation method [14]. The method for TLC and HPTLC development and selection of a suitable mobile phase involved several trials because of the complexity of the chemical composition of the herbals and the affinities of the components towards various solvents [15]. A large number of solvent systems were tried for distillate including different proportions of solvents in toluene: acetone: acetic acid solvent system [16]. The best resolution of it was observed by hptlc spectrophotometer in a solvent system, toluene: acetone: acetic acid (8: 0.5: 1.5) (Fig. 1). The band at Rf 0.39 and 0.58 appeared under 254 nm, brown and blue under 366 nm, light-brown under visible light without derivatization as denoted in Table 1.

After derivatization with 5 % methanol sulphuric acid reagent, it was seen as yellowish-brown under visible light. A brown band at Rf 0.56 was observed only under visible light after spray. Light-yellow under visible light and yellow after spray. After observation and tlc scanning by HPTLC equipment Rf values obtained [14,16]. The Rf of samples was compared with Rf of Plant *Boerhaavia diffusa* mentioned in Ayurvedic pharmacopeia and research articles [9,15]. The Rf value at each absorbance is presented in Table 1.



(A) (B) (C)

Fig. 1. (A) TLC fingerprints profile of roots of *B. diffusa* under 254 nm. (B) TLC fingerprint profile of roots of *B. diffusa* under 366 nm before spray. (C) Under visible light after spray with 5 % methanol - sulphuric acid reagent. all concentrations denoted by B1, B2, B3 for 5 µl, 10 µl, 15 µl

Table 1. Data of densitograms of absorption spectra of *Boerhaavia diffusa* 5 microliter spot

Peak	Components	% age area	Height	Rf
1	B1	62.7	948.11	0.39
2	B1	37.3	531.39	0.59

Table 2. Data of densitograms of absorption spectra of *Boerhaavia diffusa* 10 microliter spot

Peak	Components	% age area	Height	Rf
1	B2	44.8	825.88	0.01
2	B2	34.8	1088.39	0.39
3	B2	20.4	626.40	0.58

Table 3. Data of densitograms of absorption spectra of *Boerhaavia diffusa* 15 microliter spot

Peak	Components	% age area	Height	Rf
1	B3	30.8	982.62	0.01
2	B3	11.8	811.86	0.06
3	B3	35.3	1338.14	0.38
4	B3	22.0	821.06	0.58

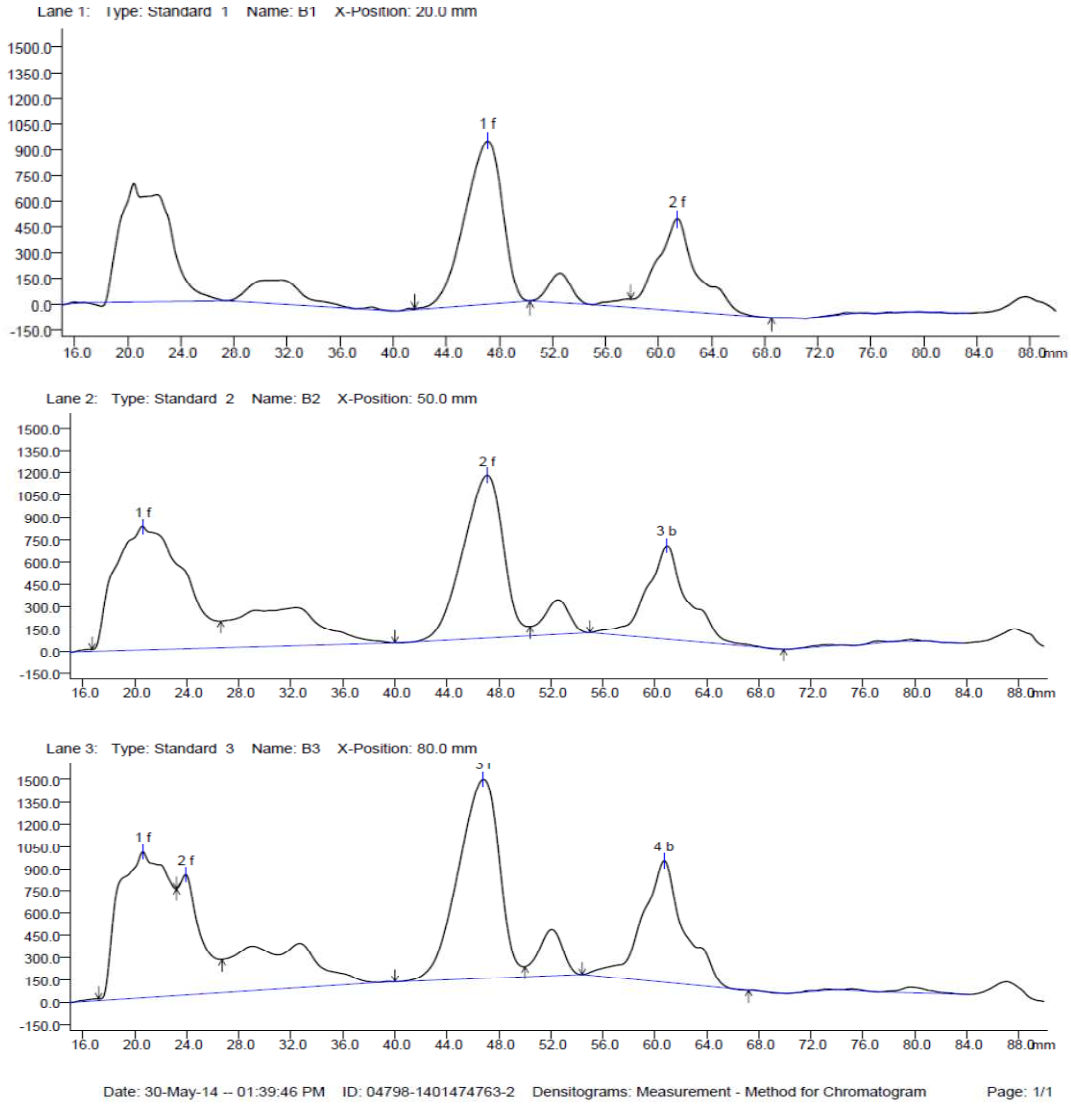


Fig. 2. Densiogram peaks of B1, B2, and B3 for 5 µl, 10 µl, 15 µl as 1f, 2f and 3f at 254 nm UV range of *Boerhaavia diffusa* aq. distillate. Rf values were calculated by observing highest height of spots appeared in HPTLC. 1f (948.11) 2f (1088.39) and (1338.14) contained same Rf values range 0.38 -0.39

4. CONCLUSION

A TLC densitometric method for the quantification of *Boerhaavia diffusa* aqueous distillate, from roots using HPTLC was developed and validated. The method was found to be simple, precise, specific, sensitive and accurate and can also be used for the quantification in the herbal raw materials. It can also be used in routine quality control of herbal materials as well as formulations containing any or both of these compounds.

5. FUTURE SCOPE

The constant Rf values by above three samples of *Boerhaavia diffusa* aqueous distillate, indicates the prominent presence of the chemical constituents which will be used to establish its quantitative presence in isolation of new compound and for its formulation development.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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