

## HPTLC METHOD DEVELOPMENT FOR THE ESTIMATION OF EUPALITIN-3-O-B-D-GALACTOPYRANOSIDE FROM TABLET AND HERB

Ashvini Maval\* and Dr. A. V. Baviskar

Department of Quality Assurance, PRES's College of Pharmacy (For Women), Chincholi,  
Nashik, MS, India.

Article Received on  
06 March 2021,

Revised on 27 March 2021,  
Accepted on 16 April 2021

DOI: 10.20959/wjpr20215-18736

### \*Corresponding Author

Ashvini Maval

Department of Quality  
Assurance, PRES's College  
of Pharmacy (For Women),  
Chincholi, Nashik, MS,  
India.

### ABSTRACT

A simple, rapid, selective and quantitative HPTLC method has been developed for determination of Eupalitin-3-O-B-D-galactopyranoside in Punarnava extract and Punarnava tablet formulation. The alcoholic extract of *Boerhavia diffusa* and its ayurvedic formulation-Himalaya Punarnava tablet samples were applied on TLC Aluminium plate pre coated with Silica gel60 GF254 and developed using Ethyl acetate: Methanol:Formic acid (5:0.5:0.5) v/v as a mobile phase. Quantification was carried out densitometrically using an UV detector at wavelength of 366 nm. The results obtained complies the limit of assay as per I.P.

**KEYWORDS:** Punarnava, Punarnava tablet, Eupalitin-3-O-B-D-galactopyranoside, TLC, HPTLC.

### INTRODUCTION

*Boerhaavia diffusa* is a species of flowering plant in the four o'clock family which is commonly known as punarnava (meaning that which rejuvenates or renews the body in Ayurveda),<sup>[2]</sup> red spiderling,<sup>[1]</sup> spreading hogweed,<sup>[1]</sup> or tarvine.<sup>[1]</sup> It is taken in herbal medicine for pain relief and other uses. The leaves of *Boerhaavia diffusa* are often used as a green vegetable in many parts of India. Having anti-inflammatory and expectorant properties, *Boerhavia diffusa* (Punarnava) is said to be a good cure for Amavata (a disease in which reduction of Vata Dosha and accumulation of Ama take place in joint(s), and simulates rheumatoid arthritis (RA)). The root acts as an anticonvulsant, analgesic,<sup>[4]</sup> laxative medication that when rubbed in honey can be locally applied for cataract, chronic conjunctivitis and blepharitis. Useful for curing heart diseases, anemia and edema (or

oedema), Punarnava is an effective remedy that reduces swelling and foul smell in skin disorders. Apart from the root, Punarnava's leaves are also consumed as a vegetarian dish to reduce oedema. As an Ayurvedic medicine, this herb is said to cure disorders like intestinal colic, kidney disorders, cough, hemorrhoids, skin diseases, alcoholism, insomnia, eye diseases, asthma and jaundice. Boerhaavia G and Boerhavia H are two rotenoids isolated from *B. diffusa*.<sup>[6]</sup> A quinolone alkaloid, lunamarine, isolated from *B. diffusa*<sup>[7]</sup> has shown some in vitro anticancer,<sup>[8]</sup> antiestrogenic,<sup>[9]</sup> immunomodulatory,<sup>[10]</sup> and anti-amoebic activity (particularly against *Entamoeba histolytica*).<sup>[11]</sup> The plant contains a protein called BDP-30, presumably a ribosome-inactivating protein.<sup>[12]</sup>

## MATERIAL AND METHOD

### Material

Himalaya Tablet was procured from local market of Nashik, MS, India. The solvents were purchased from local market of Nashik and are of analytical grade.

### HPTLC

CAMAG Linomat 5 "Linomat5-210175" S/N 210175 (1.00.13) system was use for the study purpose.

## METHOD

### Standard preparation

Weigh exactly 750 mg of Punarnava extract. Add 5 ml of Methanol and sonicate for 10 min. Filter through anhydrous Sodium Sulphate. Collect the filtrate in 10 ml volumetric flask. Wash the filtrate with 2 ml of Methanol and collect filtrate. Make up the volume up to 10 ml mark and use for TLC Spotting.

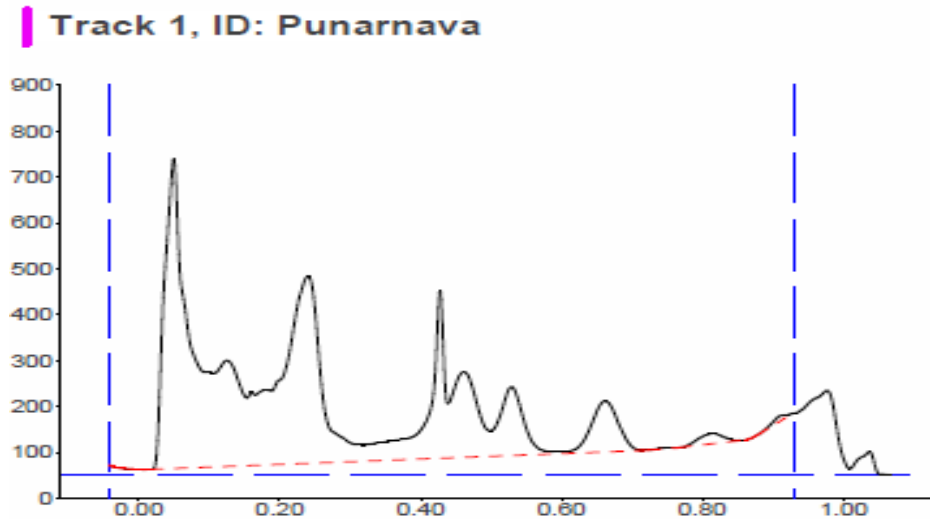
### Sample preparation

Weigh accurately tablet powdered previously equivalent to 250 mg Punarnava extract (626mg as per average wt of tablet). Add 5 ml of Methanol and sonicate for 10 min. Filter through anhydrous Sodium Sulphate. Collect the filtrate in 10 ml volumetric flask. Wash the filtrate with 2 ml of Methanol and collect filtrate. Make up the volume upto 10 ml mark and use for TLC Spotting. Scanning was done at 366 nm.

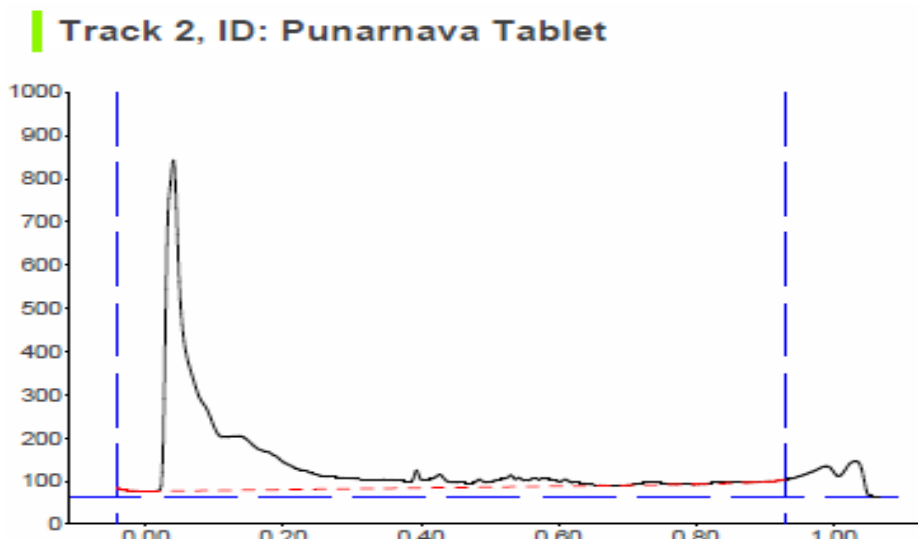
### Chromatography

TLC Aluminum pre coated plate with Silica gel 60 GF254 (20x10 cm<sup>2</sup>; 0.2 mm thick) was used with Ethyl acetate: Methanol:Formic acid (5:0.5:0.5) v/v as a mobile phase. Methanolic extract of sample and Eupalitin-3-O-B-D-galactopyranoside was applied on TLC plate using Linomat V applicator.

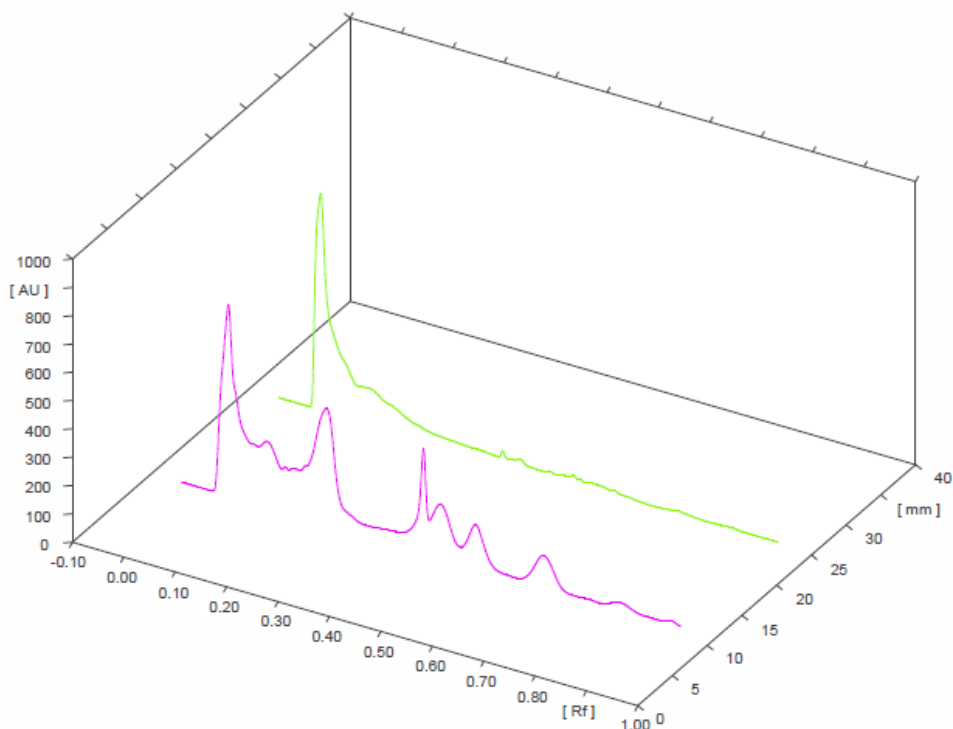
### RESULTS AND DISCUSSION



**Fig. no. 01: HPTLC chromatogram of Punarnava.**



**Fig. no. 02: HPTLC chromatogram of Punarnava tablet.**



**Fig. no. 03: Scanning chromatogram of Punarnava and Punarnava Tablet.**

**Table no. 01: As per Chromatogram RF Selected as per Reference of ICMR.**

Track no.	Wavelength (nm)	Rf value	Conc.	Peak area (AU)	Color of band
1	366	0.33	75 mg/ml	2786.3	Green
2	366	0.35	62.6 mg/ml	939.9	Green

**Calculations:** (At 366 nm)

$$\begin{aligned} \% \text{ of Punarnava} &= \text{Area of sample} \times \text{Conc. of STD} \times 100 / \text{Area of STD} \times \text{Conc. Of Sample} \\ &= 939.9 \times 75 \times 100 / 2786.3 \times 62.6 \\ &= 40.41 \% \text{ w/w} \end{aligned}$$

Avg wt of Tablet: 626 mg As per label claim Each Tablet Content 250 mg Punarnava Extract.

By Considering 626 mg Tablet =100%

X mg Punarnava Extract= 40.41 %

By cross multiplication:  $40.609 \times 626/100 = \mathbf{252.96 \text{ mg Punarnava Extract.}}$

The various mobile phases tried for Punarnava extract Ethyl acetate: Methanol: formic acid shows green band at 366nm at a Rf value of 0.33 very effectively for Punarnava tablet formulation and Punarnava extract. Sharp peaks of Eupalitin-3-O-B-D-galactopyranoside were obtained when TLC plate was scanned at 366 nm. Quantity of Eupalitin-3-O-B-D-galactopyranoside from tablet formulation and alcoholic extract was obtained automatically

and was used in formula to find out percentage purity of Eupalitin-3-O-B-D-galactopyranoside from Punarnava tablet and Punarnava extract.

## CONCLUSION

The proposed HPTLC method is simple, rapid, accurate, reproducible, selective and economic and can be used for routine quality control analysis of Eupalitin-3-O-B-D-galactopyranoside from marketed preparations of Punarnava and Punarnava plant extract.

## REFERENCES

1. Boerhavia diffusa was originally described and published in Species Plantarum 1:3. 1753. "Boerhavia diffusa". Germplasm Resources Information Network (GRIN). Agricultural Research Service (ARS), United States Department of Agriculture (USDA). Retrieved March 14, 2013.
2. Bhowmik Debjit; Kumar K. P. Sampath; Srivastava Shweta; Paswan Shravan; Sankar Amit; Dutta Dutta (2012). "Traditional Indian Herbs: Punarnava and Its Medicinal Importance" (PDF). Research Journal of Pharmacognosy and Phytochemistry, 1(1): 52–57.
3. Sherwin Carlquist (2008). "Dispersal to Islands". Plant Discoveries : Sherwin Carlquist. Retrieved March 13, 2013.
4. Kadiri Sunil Kumar; V. Saideepthi; Shaik Shahajeb; M. Nandini; R. Suthakaran (2017). "Evaluation of analgesic potential of Boerhavia diffusa roots in albino mice". Research Journal of Pharmacognosy and Phytochemistry, 9(2): 111–114.
5. Debjit Bhowmik; K.P. Sampath Kumar; Shweta Srivastava; Shravan Paswan; Amit Sankar; Dutta Dutta. "Traditional Indian Herbs Punarnava and Its Medicinal Importance"(PDF). Research Journal of Pharmacognosy and Phytochemistry – via Pharmacognosy.
6. Ahmed-Belkacem, A; MacAlou, S; Borrelli, F; Capasso, R; Fattorusso, E; Tagliatela-Scafati, O; Di Pietro, A (2007). "Nonprenylated rotenoids, a new class of potent breast cancer resistance protein inhibitors". Journal of Medicinal Chemistry, 50(8): 1933–8.
7. "Punarnavine". Comparative Toxicogenomics Database. Salisbury Cove, Maine: Mount Desert Island Biological Lab. March 6, 2013. Retrieved March 13, 2013.
8. Manu K.A.; Kuttan G. (2009). "Punarnavine induces apoptosis in B16F-10 melanoma cells by inhibiting NF-kappaB signaling". Asian Pacific Journal of Cancer Prevention, 10(6): 1031–1037.

9. Sreeja S.; Sreeja S. (2009). "An in vitro study on antiproliferative and antiestrogenic effects of *Boerhaavia diffusa* L. extracts". *Journal of Ethnopharmacology*, 126(2): 221–225.
10. Manu K.A.; Kuttan G. (2009). "Immunomodulatory activities of Punarnavine, an alkaloid from *Boerhaavia diffusa*". *Immunopharmacology and Immunotoxicology*, 31(3): 377–387.
11. Sohni YR.; Kaimal P.; Bhatt RM. (Jan 1995). "The antiamebic effect of a crude drug formulation of herbal extracts against *Entamoeba histolytica* in vitro and in vivo". *Journal of Ethnopharmacology*, 45(1): 43–52.
12. Shalini Srivastava; HN Verma; Aparana Srivastava; Vivek Prasad (2015). "BDP-30, a systemic resistance inducer from *Boerhaavia diffusa* L., suppresses TMV infection, and displays homology with ribosome-inactivating proteins". *Journal of Biosciences*, 40(1): 125–135.