# Isolation and Characterization of Eupatilin from *Boerhavia Diffusa A*erial Parts

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## Abstract

*Boerhavia Diffusa* is commonly known as punarnava belonging to family nyctaginaceae and is found throughout India. Ithas been traditionally reported its asanthelmintic, diuretic, cardiac stimulant, emetic, expectorant, anti-inflammatory, febrifuge and laxative. It is also used in jaundice, anaemia, coughand bronchitis. The secondary metabolites in a pure form such as punarnavine, b-Sitosterol, Hentriacontane, tetracosanoic, hexacosonoic, stearic, arachidic acid, urosilic acid, b-ecdysone, triacontanolhave been isolated and identified previously. In the present research work a flavonoid i.e. eupatilinis isolated and characterized for the first time.
**Keywords:**flavonoid, eupatilin, punarnava, Boerhavia

## Introduction

*Boerhavia Diffusa*is commonly known as punarnava. It is indigenous of India and found throughout warmer region of the country (**Bhavprakash, 1998).**The plant is 0.75-1m in length and is perennial herb which has creeping and purple colour stem.Leaves are short petiolate, simple, opposite, and in unequal pairs, shape is ovate oblong, acute, rounded at base. Flowers are small and pink colored small short stalked, in irregular clusters of terminal panicles.Fruitsare nuts which are one seeded, round, about 1cm long. Roots are long, circular and yellowish brown to brown in colour, surface is twisted on drying. It is soft to touch but presence of minute longitudinal markings causes it to rough, root scars are present, fracture is short. Plant Flowering and fruiting during winter. It has two varieties Red Punarnava and White Punarnava(**Longman, 1994).**

The white variety of punarnava is used in anaemia, oedema, heart diseases, cough and intestinal colic (DhanwantariNighantu). The red variety is used in the conditions of oedema, haemorrhage, anemia and biliousness. In Rajnighantu the white variety is recommended in diseases of the nervous system and in Bhavprakash in heart disease and piles. Charaka used its ointment in leprosy and skin diseases and as a decoction in stone in the kidney and in oedema. Sushtra

mentioned its use in snake poisoning and rat bite infection. Chakradatta used it in the treatment it in insomnia, rheumatism and eye diseases **(Nadkarni, 2007).**

It is used as bitter, anthelmintic, diuretic, cardiac stimulant, aphrodisiac, emetic, expectorant, anti-inflammatory, febrifuge and laxative. It is also used in scabies, jaundice, anaemia, constipation, cough, and bronchitis **(Khare, 2007).** In Unani system of medicine leaves are used in ophthalmia and for joint pain. The seeds are tonic, expectorant, carminative, lumbago, scabies, scorpion sting, purify the blood, hasten delivery (Unani). The root is well known for its diuretic and expectorant effect. In large doses it is emetic. In Punjab the drug is considered for the usefulness of the eye **(Kirtikar and Basu, 2008).**The air dried plant contains a large quantities of potassium nitrate therefore it has diuretic action. Apart from this it also contain an alkaloid i. e. punarnavine, in very small quantities (0.01% w/w). It also contains of b-Sitosterol, Hentriacontane, tetracosanoic, hexacosonoic, stearic, arachidic acid, urosilic acid, b-ecdysone, triacontanoletc**(Nadkarni, 2007).**

But these studies are not enough to identify and characterize the phytochemicals present in the plant. Hence the present study initiated to isolate and characterize the constituents from *Boerhaviadiffusa*aerial parts.

## Materials and Methods

### Collection and Authentication

Aerial parts of *Boerhaviadiffusa* belonging to family nyctaginaceae were collected in the month of september from local region of Bhopal district, Madhya Pradesh (India) and were authenticated from DrVinayakNaik, Senior Research Scientist, Piramal Life Sciences India Ltd. Goregaon (E), Mumbai. A voucher specimen of the plant (No. NPIL/PLS/05-378) has been deposited for future reference.

### Extraction and isolation

All solvents used for extraction were of technical grade, which were distilled and dried before use. Solvents used for Column Chromatography and preparative TLC were of Analytical Reagent grade. Adsorbent for column chromatography was silica gel G 60-120 (Merck).

### Procedure for Extraction and Isolation

The air dried powdered aerial part (1000g) was extracted with ethanol (70%v/v) and concentrated in rotary evaporator under reduced pressure to get ethanol extract (115.4g). Ethanol extract was dissolved in ethanol and water (1:2 v/v) and partitioned with ethyl acetate in 50mL portion for several times till complete extraction takes place. Resulting ethyl acetate fraction was concentrated under reduced pressure (58.1g) and was chromatographed on silica gel column (70cmX15cm, 60-120mesh, 2kg) chromatography and preparative TLC.

Column was initially eluted with chloroform later on polarity was increased with methanol in different concentrations (100:0, 95:5, 90:10, 85:15, 80:20, 70:30, 60:40v/v). 110 fractions each of 50mL were collected and TLC was performed of each fraction individually and eluates were monitored for the presence of various constituents. Fractions were pooled on the basis of their TLC profile, pooled fractions (13-19) were selected for the isolation of constituents. Preparative TLC of isolated constituents offered BD1.

**Detection of Flavonoid**

**Shinoda test**

A small amount of compound was dissolved in chloroform and to this few drops of concentrated hydrochloric acid and pinch amount of magnesium metal was added. The pink, crimson or magenta colour indicated presence of flavonoid **(Harborne,1998; Farnsworth, 1966).**

**Analytical methods**

TLC was performed on silica gel GF $_{254}$precoated (Merck) plates. IR spectra was recorded with FTIR (Shimadzu), $^{1}H$ and $^{13}C$ spectra recorded on Bruker (300MHz and 75.4MHz) in $CDCl_3$ used TMS as internal standard. ESIMS were measured using a Q-TOF micro mass spectrometer (Waters, USA).

**Results and Discussion**

Ethanolic extract obtained was 10.51% w/w. ethyl acetate extract obtained after partitioned of ethanolic extract was 58.1g upon column chromatography of ethyl acetate fraction (13-19) yielded (17mg) of pure compound by preparative TLC.

Upon qualitative test performed on pure compounds indicated, it is of flavonoid nature.

**Structural elucidation of compound BD1**

**Physical and spectral properties of isolated compound BD1**

**Appearance:** Yellow

**Solubility:** Chloroform

**TLC (Rf value):** 0.6

**IR (KBr, in $cm^{-1}$):** 3454, 2925, 1635, 1463

**$^{1}$H NMR (300 MHz, $CDCl_3$, δ, TMS=0):** δ6.907 (1H,S), 5.284 (1H,S), 3.653 (1H,S),7.609 (1H, m), 1.060 (2H,S), 1.134 (2H,S), 1.257 (2H,S), 1.625 (1H,d, J=7.2 MHz), 1.874 (1H,S),2.046 (1H,S), 2.309 (S), 2.437 (S), 2.621(S), 2.842 (d, J= 10.8 MHz).

**$^{13}$C NMR (75Hz, $CDCl_3$, δ, TMS=0):** δ122.06 (C-1'), 121.40 (C-2'), 143.96 (C-3'), 148.90 (C-4'), 128.22 (C-5'), 124.16 (C-6'), 170.66 (C-2), 131.42 (C-3), 177.45 (C-4), 131.42 (C-6), 170.98 (C-7), 77.43 (C-7).

**Mass spectral data:** Mass spectrum of isolated compound showed molecular ion m/z 343[M+1] corresponding to the molecular formula $C_{18}H_{16}O_7$.

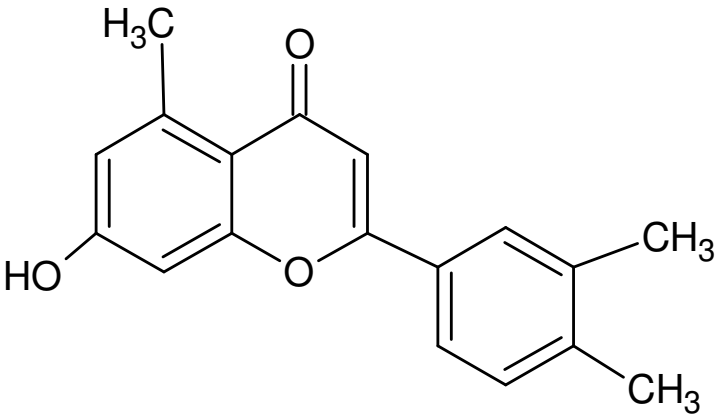


Fig 1. Chemical structure of Eupatilin

**Conclusion**: The physical, chemical and spectral evidence of compound BD1 confirms that the given constituent is eupatilin.

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