

DRUG STANDARDISATION

Boerhaavia diffusa

Pharmacognosy

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| BOTANICAL NAME | : | Boerhaavia diffusa Linn. |
| SYNONYM | : | Boerhaavia repens Linn. |
| FAMILY | : | Nyctaginaceae |
| VERNACULAR NAMES | : | Bengali : Gadhapurna, Punarnaba Raktapunarnaba English : Spreading hogweed Gujarati : Satodi, Vokhakhaparo Hindi : Biskhafra, Sant, Gadapura, Thirki Kannad : Sanadika, Komma, Kommeberu Malayalam : Thazhuthama, Titudamma Marathi : Raktavasru, Tambadivasu, Punarnava, Khapra, Vasru Oriya : Ghodapuruni Punjabi : Itsit Tamil : Mukkurattai keerai Telugu : Atikamamidi, Giligeru, Yerragolijeru Urdu : Bashkhira [1]. |

History & Authority:

The drug was proved by Ghose, S. C. [2].

Part Used:

Whole plant [3].

Distribution:

Throughout India as a weed in wastelands and roadsides, upto an altitude of 2000 m in the Himalayas [1].

Microscopical:

Stem:

Transverse section shows single layered epidermis with thick cuticle and stomata, occasionally containing yellow or reddish-brown tanniniferous contents; trichomes glandular, consisting of uniseriate, 3-10 celled stalk, 1-2 celled base and unicellular clavate or ellipsoidal head; hypodermis 1-2 layered, collenchymatous; cortex 6-7 layered, chlorenchymatous; endodermis distinct; pericycle of small isolated strands of fibres; stele polyfascicular, siphonostele and shows anomalous secondary growth; vascular bundles conjoint, collateral, endarch, in three rings: two large bundles facing each other in the center on the two sides of the pith surrounded by a second ring of 6-14 bundles and third peripheral ring (formed after secondary growth) of 15-20 conjoint, collateral and open bundles, each consisting of phloem on the outer and secondary xylem towards inner side, embedded in thick walled conjunctive tissue; bundles of raphides of calcium oxalate and starch grains scattered in parenchymatous tissue [6,7,8,9,10,11,12] (Fig. 3-5).

Description:

A variable, diffusely branched, perennial herb. Root stock stout, fusiform, woody. Stems terete, creeping, slender, prostrate or ascending, purplish, swollen at the nodes, pubescent or glabrous, upto 1.2 m long. Leaves rather thick, arranged in unequal pairs at each node, long petioled, 1-4 cm long, ovate-oblong or sub-orbicular, green, glabrous above, usually white beneath, base rounded or sub-cordate, margins subundulate, entire or sinuate. Flowers minute, sub-capitate, 4-10 together, red, pink or white, in small bracteolate umbels, forming slender, long stalked axillary and terminal panicles; bracteoles lanceolate, acute; perianth 3 mm long, ovarian part of the perianth tube 1.3 mm long, contracted above the ovary, glandular-viscid, limb funnel shaped; stamens 2 or 3, scarcely exerted. Fruits ovate, oblong, pubescent, five-ribbed, viscid, with glandular anthocarp [1,3,4,5].



Fig. 1 : *Boerhaavia diffusa* Linn.



Fig. 2 *Boerhaavia diffusa* Linn. (Flower)

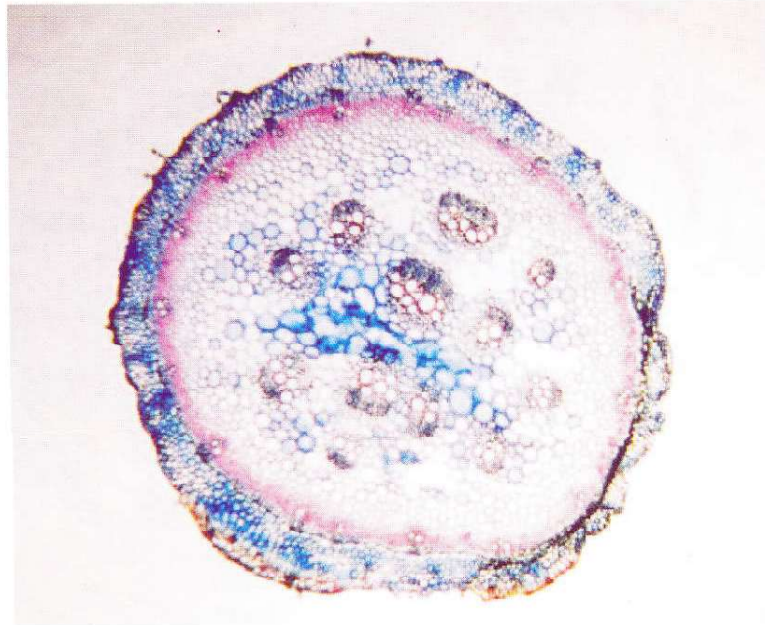


Fig. 3 : Transection of stem, under low power showing arrangement of tissues (x26).

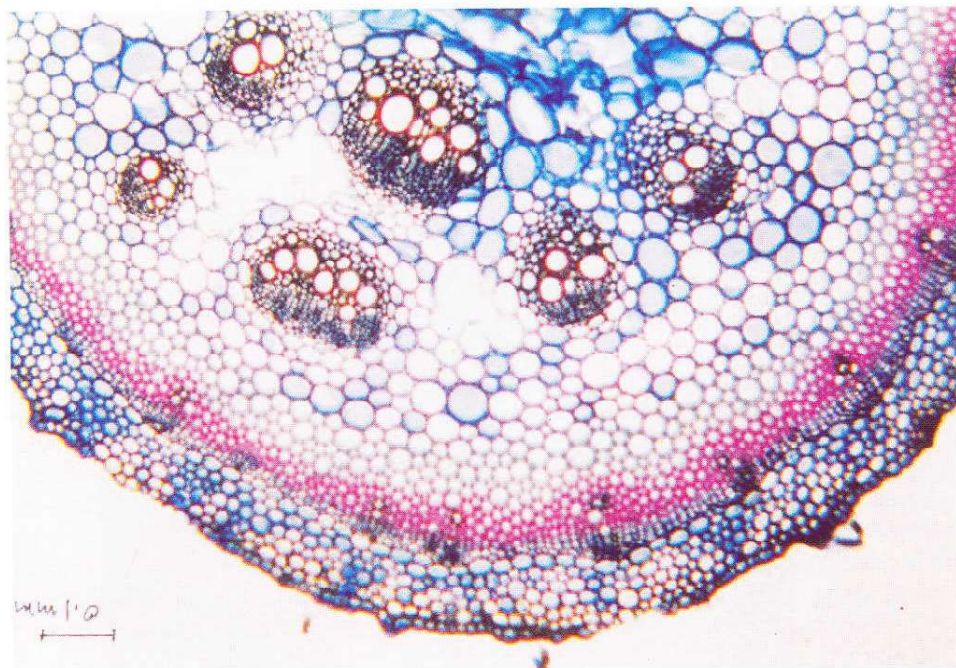


Fig. 4 : Transection of stem ,a portion magnified showing arrangement of tissues (x65).



Fig 5: Surface view of stem epidermis under high power showing glandular trichomes (x260)

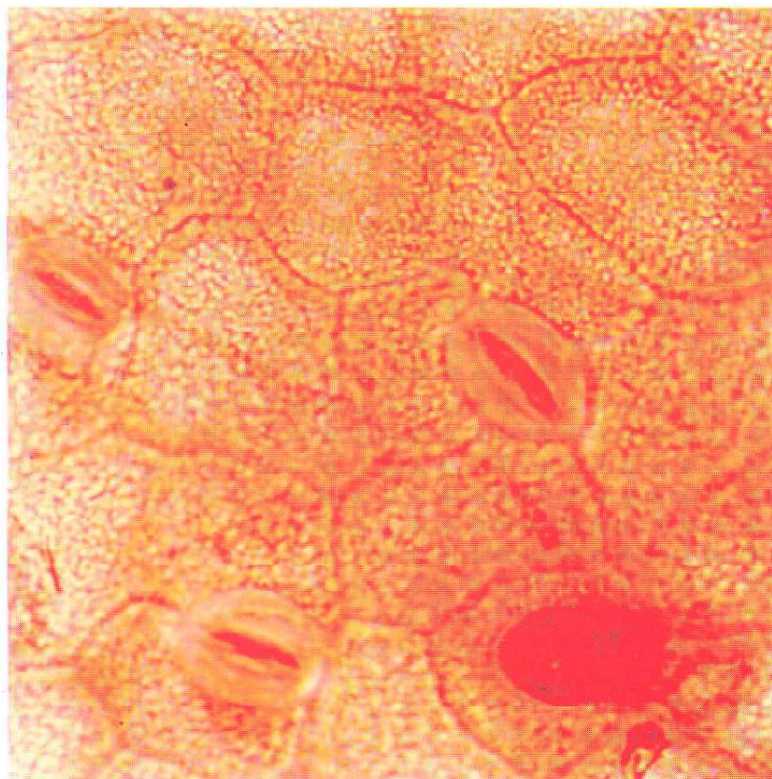


Fig 6: Surface view of abaxial epidermis showing stomata (x446).

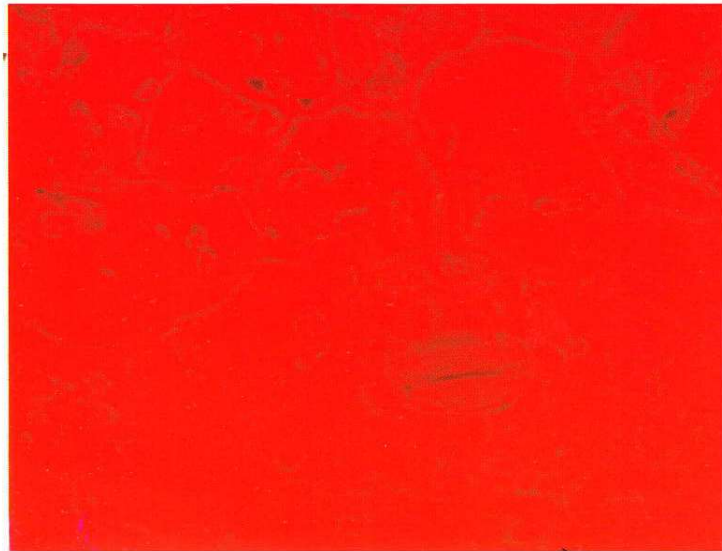


Fig 7: Surface view of adaxial epidermis showing stomata (x446).

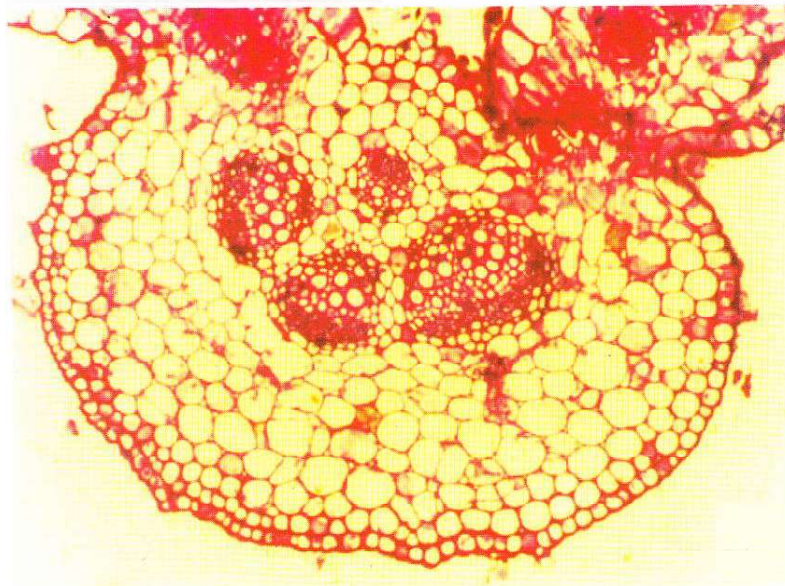


Fig 8: Transection of leaf through midrib region showing arrangement of tissues (x156).

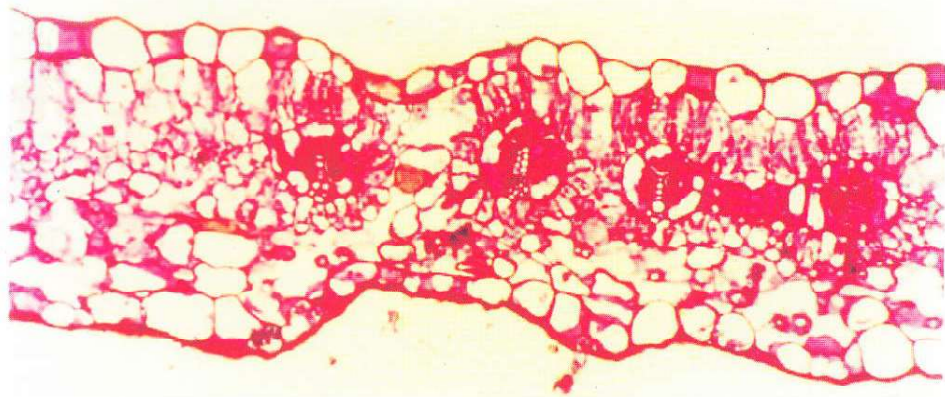


Fig 9: Transection of leaf through laminar region showing arrangement of tissues (x108).

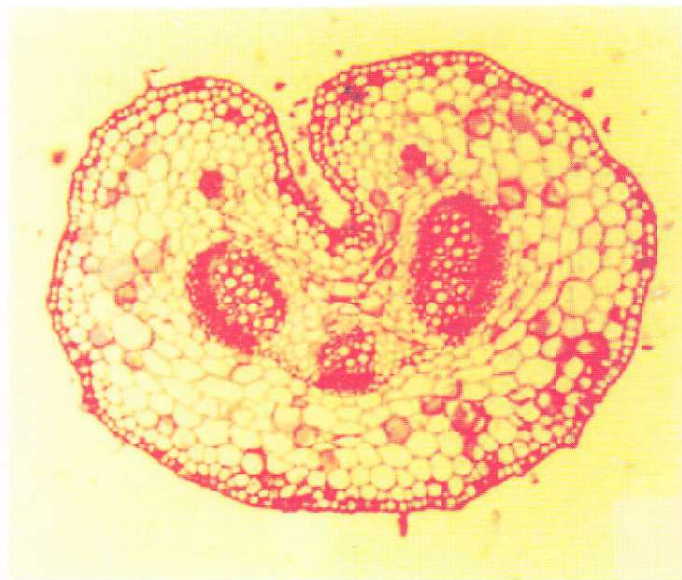


Fig 10: Transection of petiole (x49.7).



Fig 11: Transection of root under low power showing arrangement of tissues (x26).

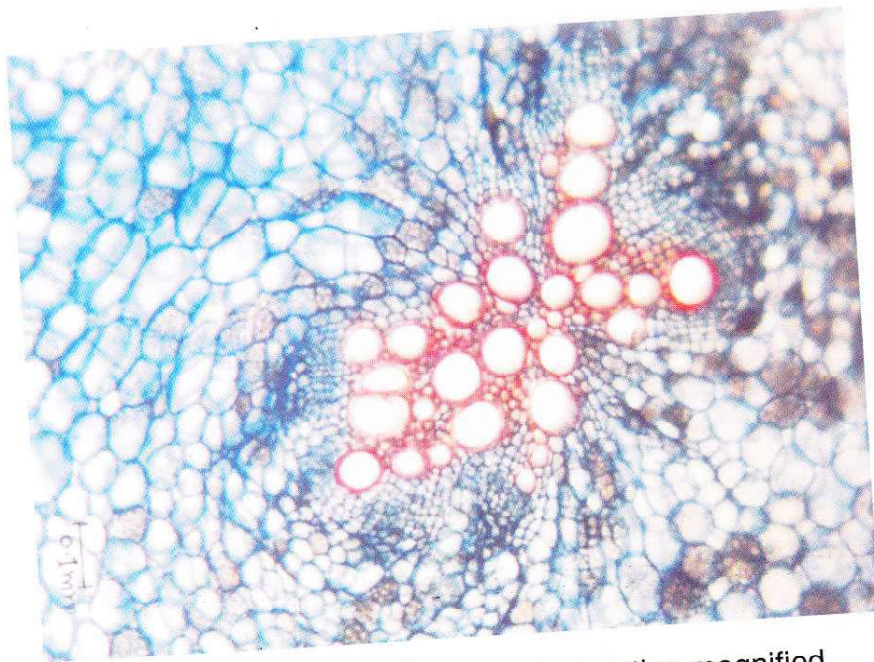


Fig 12: Transection of root, central portion magnified showing primary xylem (x65).

Leaf:

Dorsiventral; transverse section shows single layered epidermis with granular crystals, appear polygonal with straight anticlinal walls in surface view, cuticle thick, anomocytic stomata on both the surfaces; trichomes numerous as in stem; mesophyll differentiated into 1-3 layers of palisade and 3-4 layers of spongy parenchyma; vascular bundles of smaller veins surrounded by sheath of large parenchymatous cells, except opposite phloem; midrib shows 3-4, separate, conjoint, collateral vascular bundles forming an arc towards lower surface and a small vascular bundle on upper surface having phloem towards epidermis; some of the parenchyma cells of mesophyll contain bundles of raphides and occasionally clusters of calcium oxalate crystals. Palisade ratio 3.5-6.5 and stomatal index 11 to 16 (Fig.6-9).

Petiole:

Petiole in transverse section shows circular outline with an adaxial groove. Epidermis single layered, covered with thick striated cuticle; stomata on adaxial side; trichomes similar to leaf all over the surface; hypodermis single layered, collenchymatous, 2-3 layered in the adaxial ridges; vascular bundles 3-7, separate, conjoint, collateral, encapped by sclerenchymatous sheath on both sides and embedded in parenchymatous ground tissue; bundles of raphides and cluster of calcium oxalate crystals similar as in leaf [9,11,12,13] (Fig.10).

Root:

Transverse section shows cork of thin walled rectangular cells; cork cambium 5-6 layered; secondary cortex parenchymatous; stele exhibits successive rings of secondary, conjoint, collateral, vascular bundles embedded in parenchymatous ground tissue; outermost 1-2 rings of vascular bundles embedded in interfascicular conjunctive tissue; primary xylem diarch at the center; vessels with spiral and scalariform thickenings; pith absent; bundles of raphides and starch grains occasionally present in parenchyma [9,11,12] (Fig 11-12).

Powdered Drug:

Light green, coarse, with characteristic odour; shows presence of glandular trichomes with multicellular, uniseriate, 3-12 celled stalk and unicellular, clavate or ellipsoidal head; parenchyma cells of different shapes and sizes; fragments of palisade cells; bundles of raphides; numerous simple and compound starch grains; vessels with spiral and scalariform thickenings; thick walled epidermal cells with anomocytic stomata [11,12].

Qualitative Tests:

The alcoholic extract of raw drug was subjected to following phytochemical tests.

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| 1. Molisch test | : | Glycosides | +ve |
| after hydrolysis | | | |
| 2. Mayer's reagent | : | Alkaloid | +ve |
| 3. Leibermann -Buchard reaction | : | Sterol | +ve |

Physico - Chemical Studies**Raw Drug Standardization:**

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| 1. Loss of weight on drying of air dried material at 105 | : | 9.5% w/w. |
| 2. Total ash | : | Not more than 12.5% w/w. |
| 3. Acid insoluble ash | : | Not more than 4% w/w. |
| 4. Sulphated ash | : | Not more than 1.3% w/w. |
| 5. Extractive values in: | | |
| a). Petroleum ether | : | 1 - 1.5% w/v. |
| b). n-Hexane | : | 1.5 - 1.8% w/w |
| c). Chloroform | : | 0.46 - 0.55% w/w. |
| d). Ethyl acetate | : | 0.8 - 1.0% w/w |
| e). Acetone | : | 1 - 1.3 w/w. |
| f). Absolute alcohol | : | 10.3 - 13% w/w. |
| f). Purified water | : | 20 - 22% w/w. |

Preparation Of Mother Tincture:

Mother tincture was formulated as per Homoeopathic Pharmacopoeia of India by percolation method [3].

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| Drug strength | : | 1/10 |
| Boerhaavia diffusa in coarse powder containing solid 100 g. and plant moisture 9.5 ml | : | 109.5 g. |
| Purified water | : | 400 ml. |
| Strong alcohol | : | 635 ml. |

To make one thousand milliliters of the mother tincture.

Standardisation Of Mother Tincture.

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| 1. Organoleptic properties: | | |
| a). Appearance | : | Clear non viscous. |
| b). Colour | : | Greenish |
| c). Odour | : | No Characteristic odour |
| 2. Sediments | : | Absent. |
| 3. Wt. per ml. | : | from 0.910 to 0.945 gm. |

4. Total solids : Not less than 0.74% w/v.
5. Alcohol content : 57 to 61.0% v/v.
6. pH at R.T. : 6 - 6.5
6. λ max : 272 and 332 nm.

Identification:

1. TLC of mother tincture was carried out using chloroform: methanol (9:1 v/v) as mobile phase. A) Under U.V. light, five spots appeared at Rf. 0.60 (red) 0.68, 0.78, 0.85 (all blue) and 0.96 (red). B). On spraying the plate with Vanillin-sulphuric acid 8 spots appeared at 0.12, 0.18, 0.24, 0.36, 0.50, 0.60, 0.72 and 0.96.

2. 20 ml mother tincture was evaporated on water bath to remove alcohol. The content was made alkaline with 10% Ammonia solution and extracted with 2x20 ml chloroform. The chloroform extract was concentrated 2 ml and was used for TLC using n-butanol : acetic acid : water (4:1:1 v/v) as mobile phase. On spraying with Dragendroff's reagent 3 spots appeared; one prominent orange spot at Rf. 0.4 and two small spots at Rf 0.5 and 0.7.

Chemical Constituents:

Punarnavoside, rotenoids viz. Boeravinones A, B, C, D and E; lignans viz. ligidodendrin and syringaresinol mono- β - D glucoside; flavones and sterols, an isofuroxanthone; boeravine and hypoxanthine - 9 - L-arabino furanoside have been reported [14].

Pharmacological Studies:

Boerhaavia diffusa seeds are considered to be tonic, expectorant, carminative; useful in muscular pain and scabies. Leaves are employed as appetizer, alexiteric; used in wounds of eye and pain of the joints. The root possessed diuretic, anticolvulsant and analgesic properties. In traditional medicine, it is used for its anti-inflammatory, anti-bacterial and cardiotoxic properties. It is also used in the treatment of elephantiasis [4, 15].

Aqueous extract of leaves of Boerhaavia diffusa exhibited hypoglycaemic effect in alloxan-induced diabetic rats [16], whereas, aqueous extracts of its root showed hepato-protective activity in thioacetamide intoxicated rats [17] and anti-viral activity [18]. Aqueous extracts of both (roots and leaves) exhibited significant diuretic and anti-inflammatory activity in rats [19]. It also lowered uric

acid concentration in albino rats and chicks [20]. The alkaloid fraction isolated from Boerhaavia diffusa has reversed the depleted adrenal cortisol level and elevated plasma cortisol level in the stressed rats [21].

Animal Experimentation:

Urolithiasis - Study On Urinary Calculi.

A pilot study was undertaken to evaluate the efficacy of Boerhaavia diffusa mother tincture (Q) on experimentally induced urinary calculi in albino rats.

The urinary calculi induced experimental rats were divided into three groups, each groups having 5 rats and received 0.2 ml/100 gm body weight Boerhaavia diffusa Q, 60% alcohol and 0.9 % physiological saline once a day for two weeks. After 15 days, rat of all the 3 groups were transferred to metabolic cages for total urine collection in 24 hours. Volume of collected urine was noted and output in 24 hours for each rat was calculated. Specific gravity and microscopical examination was also done in addition to the estimation of excretion of calcium, phosphorus, creatinin and blood urea.

It is inferred that Boerhaavia diffusa Q at a dose level of 0.2 ml/100 gm. body weight exhibited mild increase in urinary output in 24 hours and specific gravity was noticeable in the same range having P value <0.05 when compared to control and normal control group.

It was also noticed that no traces of calcium oxalate crystals were observable in drug treated group as compared to control group. Urinary excretions of calcium, phosphorus and creatinin have no perceptible difference in test drug & control groups. Hence, preliminary studies specifically biochemical profile of urine, blood urea, specific gravity and microscopical examination revealed that Boerhaavia diffusa Q has perceptible effect in improving the urinary function.

Bibliography :

1. Anonymous. *The Wealth of India, Raw Materials*, Publication & Information Directorate, CSIR, New Delhi. 1988; 2: 174-176.
2. Ghose, S.C. *Drugs of Hindoosthan*, Hahnemann Publishing Co. Calcutta. 9th ed. 1980 ; 93-102.
3. Anonymous. *Homoeopathic Pharmacopoeia of India*, Govt. of India, New Delhi. 1971; 1: 78.

4. Kirtikar, K.R. and Basu, B.D. *Indian Medicinal Plants*, Oriental Enterprises, Dehradun. 2001; 9: 2816-2820.
5. Babu, C.R. *Herbaceous Flora of Dehradun*, Publication and Information Directorate, CSIR, New Delhi. 1977; 423-424.
6. Anonymous. *Medicinal Plants of India*, ICMR, New Delhi. 1976; 1:139-144.
7. Metcalfe, C.R. and Chalk, L. *Anatomy of the Dicotyledonous, wood structure and conclusion of the general introduction*, Clarendon Press, London. 2nd ed., 1989; 2: 84-85.
8. Saxena, A.K. and Sarabhai, R.P. *A Text Book of Botany*, Kitab Ghar, Gwalior. 1970; 3: 509.
9. Sharma, P.C.; Yelne, M.B. and Dennis, T.J. *Database on Medicinal Plants used in Ayurveda*, CCRAS, New Delhi. 2000; 1: 360-377.
10. Dutta, A.C. *Botany*, Oxford University Press, Calcutta. 3rd ed., 1970; 253-254.
11. Anonymous. *Technical Report, Drug Standardisation Unit (H)*, CCRH, Ghaziabad. 2006
12. Anonymous. *Technical Report, Drug Standardisation Unit (H)*, CCRH, Hyderabad. 2006
13. Anonymous. *Annual Report, Drug Standardisation Unit (H)*, CCRH, Patna. 1980-81; 13.
14. Sharma, P.C., Yelne, M.B. and Dennis, J. S. *Database on medicinal plants used in Ayurveda*, CCRAS, Govt. of India, New Delhi. 2001; 1: 360.
15. Nadkarni, A.K. *Indian Materia Medica*, Popular Prakashan, Bombay. 1976; 1: 202.
16. Chude, M.A., Orisavakva, O.E., Afonne, K.S., Gamaiel, O.H and Vongtav, O.J., and Obi, E. Hypoglycaemic effect of the aqueous extracts of *Boerhaavia diffusa* leaves. *Indian J. Pharmacol.* 2001; 33: 215-216.
17. Rawat, A.K., Mehrota, S., Tripathi, S.C. and Shome, U. Hepatoprotective activity of *Boerhaavia diffusa* Linn. Roots - a popular Indian - ethnomedicine. *J. Ethnopharmacol.* 1997; 56: 61-66.
18. Verma, H.N., and Awasthi, L.P. Occurrence of a highly antiviral agent in plants treated with *Boerhaavia diffusa* inhibitor. *Canadian J. Bot.* 1980; 58: 2141-2144.
19. Mudgal V. et al Comparative study on medicinal activity of leaves with flowers and leaves without flowers of *Shanknapushpi* and *Punarnava*. *J. Res. Indian med. Yoga and Homoeo.* 1977; 12: 108-111.
20. Ahmed, K., and Chakraborty, S. Hypouricaemic factor in *Boerhaavia diffusa* Linn (*Punarnava*) *J. Biol. Agric. Sci.* 1961; 4: 92-93.
21. Mungantiwar, A.A., Nair, A.M., and Shinde, V.A.. Effect of stress on plasma and adrenal cortisol levels and immune responsiveness in rats: modulation by alkaloidal fraction of *Boerhaavia diffusa*. *Fitoterapia*, 1997; 68: 498-500.