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Differential Accumulation of Rotenoids in the Roots of *Boerhaavia diffusa* L. Collected from Different Locations

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Abstract : The bioactive principal, rotenoid, from the roots of *Boerhaavia diffusa* collected from various locations of NCT Delhi was extracted using organic solvents and determined with HPLC and compared with the standard isoflavenoid (rotenone). Six locations were selected for the study: Tuglaqabad, Dhaulakuan, Ridge area (Delhi University, North Campus), Nangloi, Saidulajab, Jawaharlal Nehru University area. Roots of the same age were collected for the comparison. Considerable variation was observed in the rotenoid content of the roots collected from different locations.

Key words : *Boerhaavia diffusa*, Rotenoid, HPLC, Variation

Introduction

Boerhavia diffusa is known as purarnava in the Ayurvadic literature. It is a diffuse herb with a woody rootstalk and is distributed all over India. The whole plant including the root is used for medicinal purposes. The plant exhibits somewhat periodic efficacy, with its maximum activity in the month of May (Rawat *et al*., 1997). The extracts are found to contain β sitosterol, α 2 sitosterol, palmitic acid, ester of β-sitosterol, tetracosanoic, hexacosanoic, stearic acids. Hentriacontane, β sitosterol and ursolic acid have been isolated from roots of *B diffusa*. Six new rotenoid analogues (Boeravinone A to F) have been isolated from this plant (Kadota *et al*., 1988a, b; Lami *et al*., 1991). The total alcoholic extract of *B diffusa* was found to have anti-inflammatory activity on carrageen induced oedema. The aqueous and acetone extract also showed significant anti-inflamatory activity on carrageen induced oedema and formaldehyde induced arthritis. The drug in the form of a powder or an aqueous decoction was found to be useful in the treatment of nephritic syndrome. The drug also induced diuresis, relief in clinical oedema, decrease in albumin urea, rise in serum protein and fall in serum cholesterol level. *B. diffusa* is used in traditional medicine for its anti- inflammatory, anti bacterial, and cardiotonic properties. It is used in the treatment of elephantiasis, night blindness and corneal ulcer. Some reports suggest that extracts of *B. diffusa* had a hypoglycemic effect probably due to glycosides, flavanoids, tannins and saponins present in it (Chude *et al*., 2001).

The roots available in the market exhibited varying levels of rotenoid. The observed variation could mainly be because the roots collected from wild vary in size (probably age of root), location of collection and season of collection. The present study was undertaken to study the influence of location of collection on the accumulation of the bioactive principal. In a recent review Figuieredo *et al*. (2008) have suggested that accumulation of secondary metabolites is affected by several factors such as (i) physiological variations, (ii) environmental conditions, (iii) geographic variations, (iv) genetic factors and evolution, (v) political/social conditions, and also (vi) amount of plant material/space and manual labour needs.

Materials and Methods

Root samples were collected from six locations of NCT Delhi: Tuglaqabad, Dhaulakuan, Ridge area (Delhi University, North Campus), Nangloi, Saidulajab, Jawaharlal Nehru University area. Roots were collected during the month of May and June and care was taken to select roots of the same size with respect to length and girth. Roots were then air dried, cut into small pieces and pulverized into fine powder to facilitate extraction.

Extraction (Kadota *et al*., 1989): One Kg of the root powder was cold extracted in ether three times with each extraction lasting for two days. The three extractions were pooled and concentrated in a Rotary evaporator (Buchi Rotavapor, R-124, Switzerland) to yield 200 ml of the extract and a white powder which was stored for later studies. Ether in the extract was

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further evaporated to yield a yellow brownish residue. This was redissolved in ether and partitioned with aqueous sodium carbonate. The ether fraction was collected and washed with saturated NaCl. The ether was then evaporated to get a greasy residue, which was analyzed using HPLC for rotenoids.

HPLC determination of rotenoids: The fingerprint chromatogram of the crude extract was performed by HPLC. The HPLC conditions for rotenoid were mobile phase: 60% methanol and 40% water, flow rate: 2.0 ml/min, column: µ-Bondapak C18 (30 cm x 3.9-mm ID stainless steel); ambient temperature and UV detector UV @ 290 nm; 0.1A full-scale; 1-cm cell (Anonymous, 1994). Amounts of rotenoids in each extract were analysed by comparing the HPLC finger print chromatogram of the extracts with the standard isoflavonoids (rotenone) from Sigma Co (St Louis, USA). The rotenoid in the various root extracts was calculated from a standard curve obtained from the serial dilutions of the rotenone standard. Content of this compound was calculated in percentage and mg/kg of the dried root.

Results

The HPLC profiles of the rotenone standard and the rotenoids in the semicrude extracts from the root samples collected from six locations is given in Fig 1. It was observed that under the conditions followed the rotenone standard had a retention time between 5 & 6 min. this fingerprint was used to identify rotenoids in the semi-crude root extracts. There was considerable variation in the rotenoid content of *Boerhaavia diffusa* root samples collected from various locations of NCT Delhi ranging from 5.1 to 41.3 mg/Kg (Table 1). The least amount of 5.1 mg/Kg was observed in the roots collected from Tuglaqabad region. Roots collected from the Dhaulakuan, the Delhi University Ridge area and Nangloi showed the presence of 12.5 and 16.9, and 21.5 mg/Kg of rotenoid, respectively. The maximum quantity of rotenoid was observed in the roots collected from Saidulajab (31.1 mg/Kg) and Jawaharlal Nehru University region (41.3 mg/Kg).

Discussion

The present study amply demonstrates that HPLC can be effectively used to determine rotenone in the semi-crude extracts of the roots of *Boerhaavia diffusa*. Further, it has been revealed unambiguously that location where a medicinal plant grows has considerable influence on the accumulation of the secondary metabolite. The regions selected are distinct from one another with respect to the soil conditions. The Dhaualakuan and the Delhi University ridge region are two ends of the same Ridge so the variation in the rotenoid content is not much significant. Saidulajab and Jawaharlal Nehru University area are part of the Aravali tail and so the soil composition must be similar. Both regions are rocky and dry and that probably accounts for the similar amounts of rotenoid in the roots collected from these regions. Tuglaqabad and Nangloi are distinct areas in the south and east of Delhi and both exhibit difference in the rotenoid content.

Influence of age and location on the accumulation of isoflavenoids and miroestrol has been observed in *Butea superb* and *Pueraria mirifica* growing in Thailand (Manosroi and Manosroi, 2005). In *Arnica montana*

Table 1: Accumulation of rotenoid in the roots of *Boerhaavia diffusa* collected from different locations of NCT Delhi.

Location	Rotenoid (as % of dried root)	Flavenoid as mg/Kg of the dried root
Tughlaqabad	0.00051	5.1
Dhaulakuan	0.00125	12.5
Ridge area, Delhi University (North Campus)	0.00169	16.9
Nangloi	0.00215	21.5
Saidulajab	0.00311	31.1
Jawaharlal Nehru University	0.00413	41.3

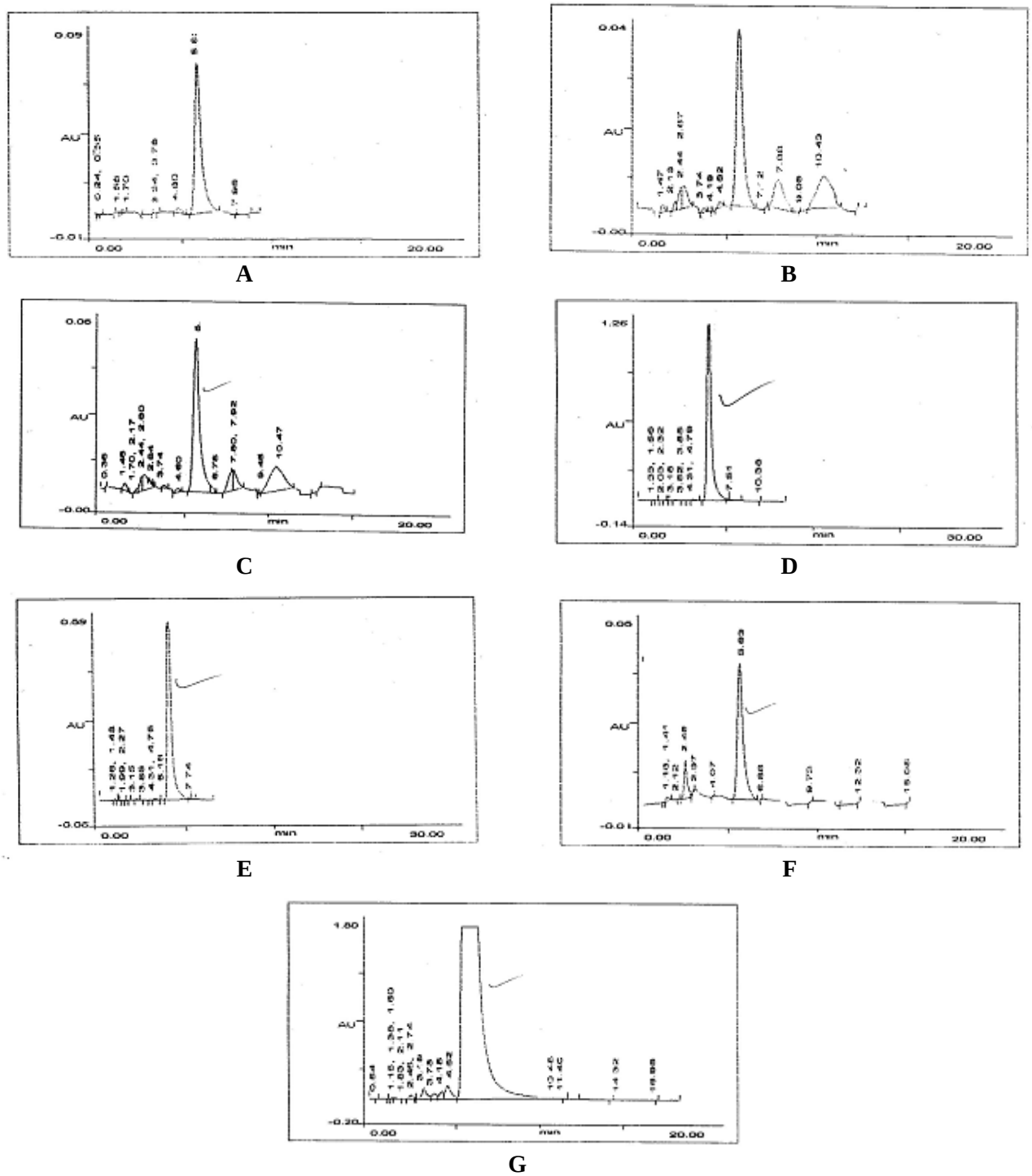

Fig. 1: HPLC profiles of rotenoid standard (A) and in the semi-crude extract of root samples collected from ix locations of NCT Delhi: Tuglaqabad (B), Dhaulakuan (C), Ridge area (Delhi University, North Campus) (D), Nangloi (E), Saidulajab (F), Jawaharlal Nehru University area (G).

altitude of the growing site positively affected accumulation of flavenoids with vicinal free hydroxyl groups in ring B and caffeic acid derivatives (Spitaler *et al*., 2006). The present work on *Boerhaavia diffusa* is in complete agreement with the observations of Figuieredo *et al*. (2008) that secondary metabolite accumulation is influenced by environmental factors.

This study indicated that the rotenoid content in the roots of *Boerhaavia diffusa* depended on locations where it is growing. This information will be beneficial for the selection of the plants collected from different localities.

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