UKaaZ

Annals of Phytomedicine 6(1): 83-87, 2017
DOI: 10.21276/ap.2017.6.1.12 ; Print ISSN : 2278-9839 and Online ISSN : 2393-9885

Ann. Phytomed., 6(1):83-87 (2017)

Original article

Phytochemical studies on the roots of *Hemidesmus indicus* (L.) R. Br. ecotypes

D. Kavitha, P. Sudhakar and P. Ramachandra Reddy
Plant Anatomy and Taxonomy Laboratory, Department of Botany, Osmania University, Hyderabad-500 007, Telangana State, India

Received January 5, 2017: Revised January 29, 2017: Accepted February 9, 2017: Published online June 30, 2017

Abstract

Hemidesmus indicus (L.) R.Br. (Syn: *Periploca indica* L.) belongs to the family, Asclepiadaceae. It is also known as "Indian Sarsaparilla" and is widely recognized in traditional systems of medicine. Ecotypes of *H. indicus* are showing the significant morphological variation and collected from different type of soil conditions. Phytochemical studies like preliminary phytochemical analysis of root extracts of ecotypes have not shown variation. HPLC chromatograms of root methanol extracts in ecotypes have showed the variation in number of peaks and results were compared with standard 2-hydroxy 4-methoxy-benzaldehyde. All the seven ecotypes showed the presence of major compound, 2-hydroxy-4-methoxy benzaldehyde and its concentration is more in Type-6 (7.80 µg/mg) and less in Type-3 (2.02 µg/mg). This study identified the ecotypes with high secondary metabolite which can help herbal drug manufacturers in identification to correct raw material.

Key words: *Hemidesmus indicus* (L.) R. Br., ecotypes, HPLC, 2-hydroxy-4-methoxy benzaldehyde

1. Introduction

Hemidesmus indicus (L.) R.Br. synonym *Periploca indica* (L.) belongs to the family, Asclepiadaceae. It is commonly known as Indian Sarsaparilla and Anantamul in Sanskrit. It is an important drug of Indian system of medicine since time immemorial. During last two decades, the drug has been extensively studied for its phytochemical, pharmacological and clinical investigations and many interesting findings have been reported in various fields. The plant has long been mentioned in Indigenous systems of Medicine as blood purifier, soothes burning sensation and useful in treatment of fever and others. The roots are used as antipyretic, antidiarrhoeal, astringent, blood purifier, diaphoretic, diuretic, refrigerant and tonic (Anonymous 1986, 1997; Nadkarni, 1989). It forms an important ingredient of some Ayurvedic preparations such *as Aswagandhadi churnam, Aswagandhadi lehyam, Chandanasava* and others (Chopra *et al.*, 1956). This is a common medicinal plant, widely used in Indian systems of Medicine (Anonymous, 1997) and also an official drug in Indian Pharmacopoeia (Anonymous, 1996) and British Pharmacopoeia (Anonymous, 2003). Literature survey revealed that roots are used as antipyretic, antidiarrhoeal, astringent, blood purifier, diaphoretic, diuretic, refrigerant and tonic besides biliousness, blood diseases, dysentery, diarrhoea, respiratory disorders, skin diseases, syphilis, fever, leprosy, leukoderma, leucorrhoea, itching, bronchitis, asthma, eye diseases, epileptic fits in children, kidney and urinary disorders, loss of appetite, burning sensation and rheumatism (Mukherjee, 1953; Chopra *et al.*, 1956; Kirthikar and Basu, 1980; Anonymous, 1986, 1997; Nadkarni, 1989). Ethnobotanical studies on *Hemidesmus indicus* (L.) R.Br. revealed its benefits towards various ailments like scorpion sting, snake bite, fever (Sharma *et al.*, 1979) and as a blood purifier (Malhotra and Murthy, 1973; Sharma *et al.*, 1979; Pullaiah and Kumar, 1996). It has cooling effect and used in venereal diseases including gonorrhea (Singh and Maheshwari, 1983). Root decoction is useful for curing high fever and skin diseases (Sudhakar and Rao, 1985; Vyas, 1993).

Based on morphological variation, seven ecotypes of *H. indicus* were collected and given name as Type-1, Type-2, Type-3, Type-4, Type-5, Type-6 and Type-7 from four districts, *viz.*, Mahabubnagar, Hyderabad, Medak and Ranga Reddy from southern part of Telangana State. Morphologically, they showed significant variation in leaf size, shape, colour, venation and phyllotaxy, grown in different type of soils like black cotton soil, red sandy soil, loam soil and rocky soil. Type-1, Type-3, Type-4 and Type-7 were collected from black cotton soil, Type-2 and Type-5 were collected from red sandy soil and Type-6 was collected from loamy and rocky soils (Figure 1). Hence, the present investigation was focused on the phytochemical variation and estimation of 2-hydroxy-4-methoxy benzaldehyde concentration in given ecotypes. The study assumes importance since there is no earlier information available on these aspects.

2. Materials and Methods

Ecotypes of *H. indicus* were collected from different places of Mahabubnagar, Hyderabad, Medak and Ranga Reddy Districts in Telangana State. Collected materials were authenticated with the help of regional floras (J.S Gamble, 1967; Cooke, 1908; and K.M. Matthew, 1983). The herbarium specimens were deposited in Herbarium Hyderabadense (HY), besides; duplicates of several collections have been deposited in plant Anatomy and Taxonomy

Author for correspondence: Dr. D. Kavitha
Plant Anatomy and Taxonomy Laboratory, Department of Botany, Osmania University, Hyderabad-500 007, Telangana State, India
E-mail: kavitha.duggi@gmail.com
Tel.: +91-9885459535

laboratory, Deportment of Botany, Osmania University, Hyderabad- 500007, India. Matured roots of ecotypes of *H. indicus* were washed thoroughly with water for removing soil particles and dried under shade at room temperature (25°C) for ten days and powdered. Powder was filtered through 40╪ mesh particle size and stored in an air tight container at room temperature.

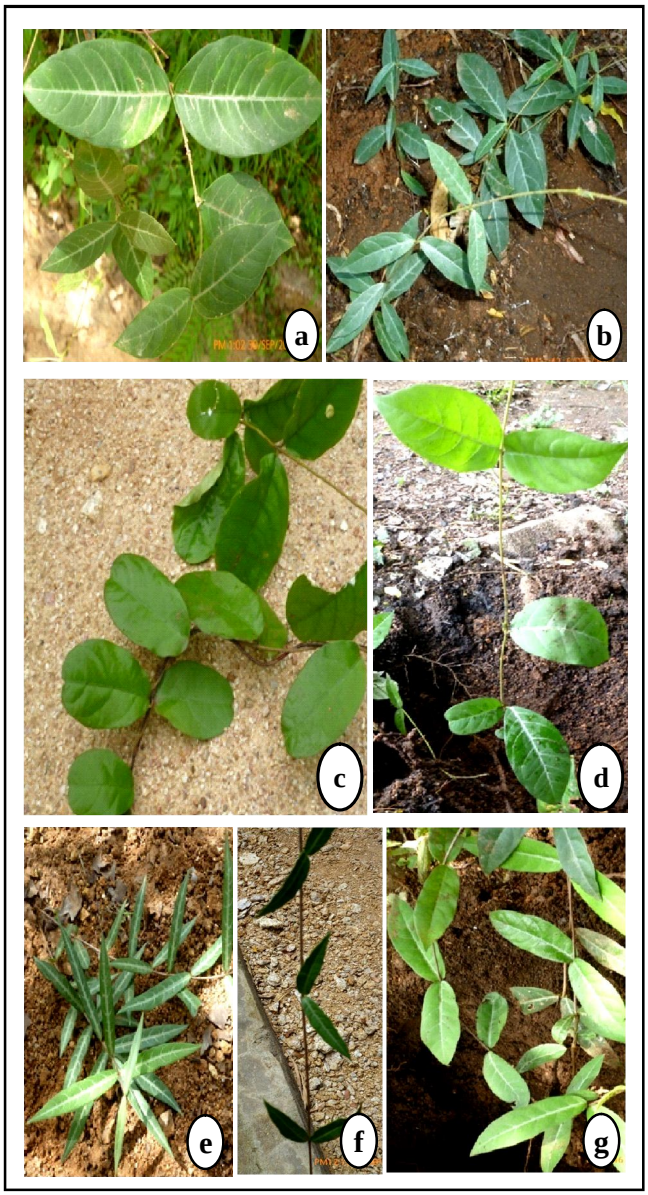

Figure1: Morphological variation in ecotypes of *H. indicus*. (a) Ovate shape leaves in Type-1, (b) Obovate-oblanceolate shape leaves in Type-2, (c) Oblong shape leaves in Type-3, (d) Ovate-ovate elliptic shape leaves in Type-4, (e) Linear lanceolate shape leaves in Type-5, (f) Lanceolate shape leaves in Type-6 and (g) Elliptic-Linear elliptic shape leaves in Type-7.

2.1 Preparation of extracts

Successive extract was carried out using Soxhlet apparatus. 25g of each ecotype root powders were extracted with 250 ml solvents like n-hexane, chloroform, acetone, methanol and water based on the order of increasing polarity. Extraction temperatures were adjusted to boiling points of solvent. The extracts were cooled and filtered through Wattmans No.1 filter paper. After the extraction, the solvents were evaporated using rotary evaporator (Heidolf®LABAROTA EficientAvaporators-4000). The crude residues were kept in refrigerator when not in use.

2.2 Phytochemical screening

Preliminary phytochemical screening was carried out by using different type of solvents to identify the major natural chemical groups such as alkaloids, flavonoids, saponins, carbohydrates, glycosides, amino acids, triterpinoids, phenols, steroids and coumarins by adopting standard procedures (Raman, 2006).

2.3 HPLC study

A HPLC system consisting of two LC-20AD pumps, an SPD-M_{20}A diode array detector (PDA), an SIL-20AC auto sampler, a DGU-$20A_5$ degasser, a CTO-20 AC column oven and a CBM-20A communications bus module (all from Shimadzu, Kyoto, Japan) were used. The data were recorded using an HP-Vectra (Hewlett Packard, Waldron, Germany) computer system using LC-Solution data acquiring software (Shimadzu, Kyoto, Japan). LCGC Qualisil BDS C18 Column (250 × 4.6mm id; 5 µm, made in USA) were used for separation. HPLC grade methanol (MEOH) (Merck, Mumbai, India), Glass-distilled and de-ionized water (Millipore, France) were used. Chromatographic separation was achieved on LCGC Qualisil BDS C-18 Column using a mobile phase mixture of MEOH: H_2O in the ratio of 70:30 (v/v) and degassed using a vacuum degasser before use. The flow rate was set at 1ml/ min and the column was maintained at ambient temperature. The injection volume was 20 µl and the detector wavelength was tuned at 280 nm.

2.3.1 Preparation of the standard 2-hydroxy-4-methoxy-benzaldehyde solution

Authentic 2-hydroxy-4-methoxy- benzaldehyde (Sigma Aldrich, 98% Purity) was used as standard. Stock solution was prepared by dissolving 1 mg crystalline 2-hydroxy-4-methoxy- benzaldehyde in 1 ml methanol in a volumetric flask. From this, working standard samples were prepared by suitable dilution of 500 ppm concentration.

3. Results and Discussion

3.1 Preliminary phytochemical analysis

The individual root extracts were subjected to the preliminary phytochemical screening for the presence of secondary metabolites. Previously, Mukherjee and Ray (1980) reported that roots of *H. indicus* to contain steroids, triterpinoids, flavonoids and saponins but alkaloids are absent. Coumarins were reported by Das *et al.* (1992). However, present study confirmed the above observations and showed the presence of alkaloids, steroids, triterpinoids, glycosides, carbohydrates, polyphenols, saponins in root extracts of seven ecotypes of *H. indicus*. In addition to this, mentioned secondary metabolites in alcoholic extract are also present in aqueous extract except alkaloids. Rajan *et al.* (2011) and Prasanna Purohit *et al.* (2014) also reported similar results (Table 1).

3.2 HPLC analysis

HPLC analysis was carried out for all the seven ecotypes of methanol root extract by using C-18 column, using a mobile phase consisting

of mixture of MEOH: H_2O in water in the ratio of 70: 30 (v/v). Results were compared with the standard 2-hydroxy-4-methoxy benzaldehyde and its presence in all the ecotypes of *H. indicus* which was confirmed when compared with the retention time (R_t). R_t of standard 2-hydroxy-4-methoxy benzaldehyde was 6.35 min whereas the same when eluted for ecotypes showed R_t 5.96 min (Type-1), 5.98 min (Type-2), 6.01 min (Type-3), 6.04 min (Type-4), 6.07 min (Type-5), 6.08 min (Type-6) and 6.13 min (Type-7). Sreelekha *et al.* (2007) reported that the fresh root on steam distillation yielded a volatile oil which contained major component of 2-hydroxy-4-methoxy benzaldehyde. Beside this, Sircar *et al.* (2007) and Kundu and Mitra (2013) reported the crude extract of *H. indicus* root to contain the high amount of 2-hydroxy-4-methoxy benzaldehyde, responsible for the sweet fragrance. Present study confirms the above observations, because peak area of the 2-hydroxy-4-methoxy benzaldehyde, compound is prominent in all seven ecotypes. But quantitatively ecotypes showed the variation. Dangi *et al.* (2012) in *Terminalia bellerica* accessions reported gallic acid percentage variation from 1.07% - 4.96%. Similarly, in the present study, concentration of the 2-hydroxy-4-methoxy benzaldehyde in root extracts are relatively higher in Type-6 (7.80 µg/mg) and low in Type-3 (2.02 µg/mg) (Table 2).

Table 1: Preliminary phytochemical analysis of roots of *H. indicus* ecotypes

Name	Hexane	Chloroform	Acetone	Methanol	Aqueous extract
Alkaloids	-	+	+	+	-
Flavonoids	-	-	+	+	+
Saponins	-	-	-	+	+
Carbohydrates	-	-	-	+	+
Cardiac glycosides	+	+	+	+	+
Proteins	-	-	-	-	+
Triterpinoids	+	+	+	-	-
Phenols	-	-	-	+	+
Steroids	-	+	-	-	-
Coumarins	-	+	+	+	+

+ = Present, - = Absent

Table 2: HPLC data for root MEOH extracts of *H. indicus* ecotypes and standard 2-hydroxy-4-methoxy benzaldehyde

Ecotypes	Retention time (R_t) in min.	Peak area	Conc. of the 2-hydroxy-4-methoxy benzaldehyde in ecotypes µg/mg
2H4MB compound standard (98% purity)	6.35	10537345	-
Type-1	5.96	12191060	5.12 µg
Type-2	5.98	1157533	3.17 µg
Type-3	6.01	5124802	2.02 µg
Type-4	6.04	7479222	2.67 µg
Type-5	6.07	15309649	7.12 µg
Type-6	6.08	16729792	7.80 µg
Type-7	6.13	5821187	2.30 µg

2H4MB: 2-hydroxy-4-methoxy benzaldehyde

HPLC chromatograms of root methanol extracts of ecotypes showed the variation in number of peaks at 280 nm. Along with the standard peak in Type-1, 2 and 4, total five peaks were observed. In Type-3, 5 and 6, total six peaks have been observed. In Type-7, total seven peaks are present. Extra peaks identified at 14.79 min R_t and 23.65 min R_t, peaks are exclusively present in Type-7 which indicate, the ecotypes may be having variation in some minor compounds (Figures 2 to 9).

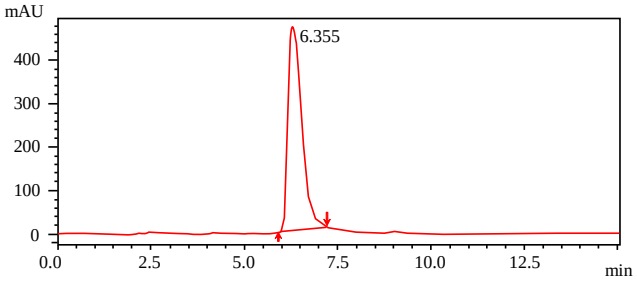

Figure 2: HPLC chromatogram of standard 2-hydroxy-4-methoxy-benzaldehyde.

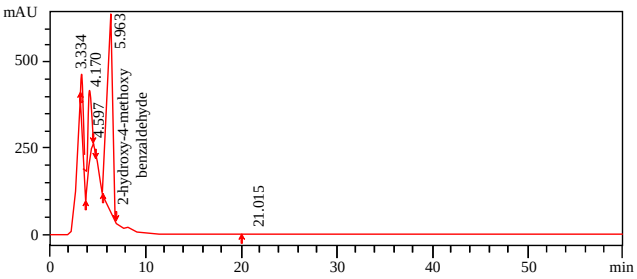

Figure 3: HPLC chromatogram of Type-1 root methanol extract.

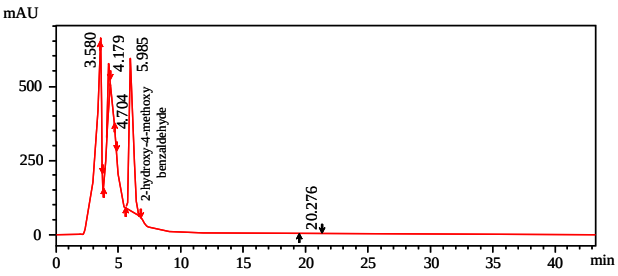

Figure 4: HPLC chromatogram of Type-2 root methanol extract.

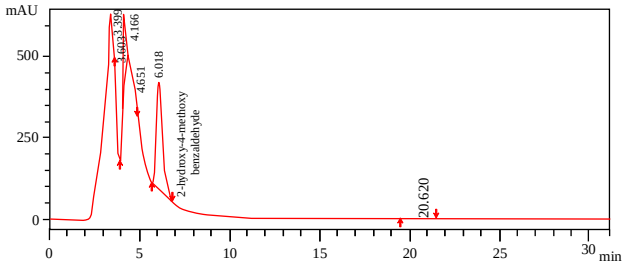

Figure 5: HPLC chromatogram of Type-3 root methanol extract.

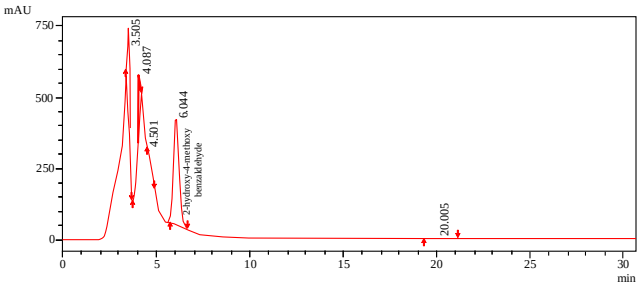

Figure 6: HPLC chromatogram of Type-4 root methanol extract.

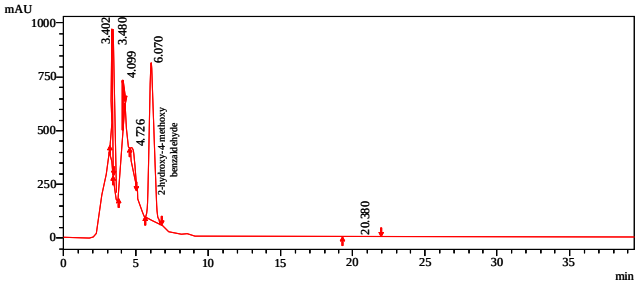

Figure 7: HPLC chromatogram of Type-5 root methanol extract.

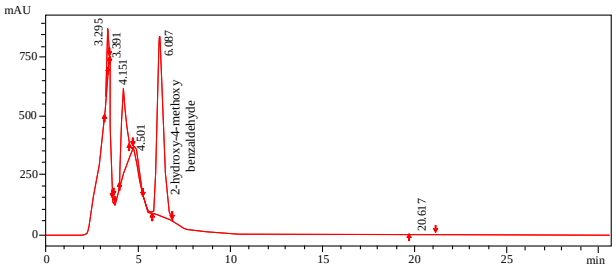

Figure 8: HPLC chromatogram of Type-6 root methanol extract.

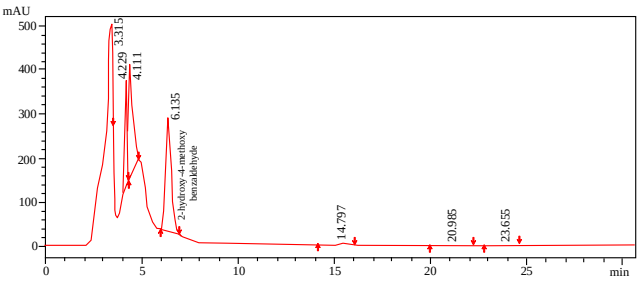

Figure 9: HPLC chromatogram of Type-7 root methanol extract.

4. Conclusion

Ecotypes of *H. indicus* showing the significant morphological variation in leaf size, shape, colour, internodal length and phyllotaxy. Therefore, the present study focused on the phytochemical variation in the given ecotypes. In preliminary phytochemical screening, all the seven ecotypes of *H. indicus* root extracts were showing the prescence of alkaloids, flavonoids, steroids, triterpinoids, glycosides, carbohydrates, phenols, saponins, proteins and coumarins. But HPLC chromatograms of root methanol extracts in ecotypes have showed the variation in number of peaks. All the seven ecotypes showed the presence of major compound, 2-hydroxy-4-methoxy benzaldehyde and its concentration is more in Type-6 (7.80 µg/mg) and less in Type-3 (2.02 µg/mg). This study identified the ecotypes with high secondary metabolite which can help herbal drug manufacturer industries.

Acknowledgements

The authors are thankful to the Head, Department of Botany, Osmania University, Hyderabad, for providing facilities and encouragement and Dr. R. Nageswara Rao (Retd), Chief Scientist, IICT for the HPLC analysis. One of us (D. Kavitha) is thankful to U.G.C (R.F.S.M.S, Letter No 94 March 2012) for providing financial support.

Conflict of interest

We declare that we have no conflict of interest.

References

Anonymous (1986). The use full plants of India, CSIR, New Delhi, India.

Anonymous. (1996). Indian Pharmacopoeia, Indian Pharmacopoeia Committee, Ministry of Health and Family Welfare, Government of India, New Delhi.

Anonymous. (1997). The Wealth of India: Raw Materials, CSIR, New Delhi, Vol. III, V and X.

Anonymous. (2003). British Pharmacopoeia. British Pharmacopia Commission, Market Towers, 1 Nine Elms Lane, London.

Chopra, I.C.; Handa, K.L. and Sobti, S.N. (1956). Need for the cultivation of vegetable drugs used in Ayurvedic and Unani medicines. Indian. J. Pharm., **18:**364-377.

Cook (1908). The Flora of The Presidency of Bombay, Vol-II: pp:146-147.

Dangi, B.; Jain, R.; Kachhwaha, S. and Kothari, S.L. (2012). Assessment of diversity in *Terminalia belerica* Roxb. using morphological, phytochemical and molecular markers. Natl. Acad. Sci. Lett., **35(1):** 27-35.

Das, P.C.; Joshi, P.C; Mandal, S.; Das, A. and Chatterjee, A. (1992). New Coumarinolignoids from *Hemidesmus indicus* R.Br. Indian. J. Chem., **31B**:342-345.

Gamble, J. S. (1967). Flora of the Presidency of Madras; Vol-II: pp:579-580.

Kirthikar, K. and Basu, BD. (1980). Indian medicinal plants. Bishen Singh Mahendra Pal Singh, Dehradun, India. Vol., pp:1-4.

Kundu, A. and Mitra, A. (2013). Flavoring extracts of *Hemidesmus indicus* Roots and *Vanilla palanifolia* pods exhibit *in vitro* Acetylcholines-terase inhibitory activities. Plant. Foods Hum. Nutr., **68:**247-253.

Malhotra, S.K. and Murthy, S. (1973). Some useful and medicinal plants of Chandrapur district (Maharashtra State). Bull. Bot.Sury. India, **15:**13-21.

Matthew, K. M. (1983). The Flora of the Tamil Nadu Carnatic, Vol-II: pp:947.

Mukherjee, B. (1953). The Indian Pharmaceutical Codex, Indigenous drugs, CSIR, New Delhi, India. Vol-II.

Mukherjee, K. and Ray, L.N. (1980). Screening of some Indian plant species. Quart. J. Crude Drugs. Res., **18:**77-82.

Nadkarni, A.N. (1989). Indian Material Medica, Popular Book Depot, Bombay, India, **1**:619.

Prasanna Purohit, Ritu Thakurm Basis, Pratibha Singh and Shagufta Khan. (2014). Assessment of antimicrobial activity of phytochemical screening of *Hemidesmus indicus* root extracts. UK. J. Pharma. Biosci., **2(6):**67-72.

Pullaiah, T. and Kumar, D.C.T. (1996). Herbal plants in Mannanur forest, Mahaboobnagar Dist., AP. In: Ethnobotany in South Asia, Maheshwari, J.K. (Ed.). Scientific Publishers, Jodhpur, India.

Rajan, S.; Shalini, R.; Bharathi, C.; Aruna, V.; Elgin, A. and Brindha, P. (2011). Pharmacognostical and phytochemical studies on *Hemidesmus indicus* root. Int. Pharmacog. Phytoche., **3**(3):74-79.

Raman, N. (2006). Phytochemical techniques, New India Publishing Agency. Pitam Pura, New Delhi.

Sharma, P.K.; Dhyani, S.K. and Shankar, V. (1979). Some useful and medicinal plants of the district Dehradun and Siwalik. J. Sci. Res. Plant Med., **1**:17-43.

Singh, K.K. and Maheshwari. (1983). Traditional phytotherapy among the tribal's of Varanasi Dist., U.P. J.Econ. Tax. Bot., **4**:803-829.

Sircar, D.; Day, G. and Mitra, A. (2007). A validated HPLC method for simultaneous determination of 2-hydroxy-4-methoxy-benzoldi-hyde and 2-hydroxy-4-methoxybenzoic acid in root organs of *H. indicus*. Chromatographia, **65**:349-353.

Sreelekha, M.; Jirovetz, L. and Shafi, P.M. (2007). Comparative study of the essential oils from *Hemidesmus indicus* and *Decalepis hamiltonii*. Asian J Chem., **19:**4942-4944.

Sudhakar, S. and Rao, R.S. (1985). Medicinal plants of upper East Godavari district (Andhra Pradesh) and need for establishment of medicinal farm. J. Econ Tax Bot.,**7:**399-405.