Original article

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

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**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, diuretic, 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, diuretic, 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 gonorrhrea (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 collections have been deposited in plant Anatomy and Taxonomy Laboratory, Department of Botany, Osmania University, Hyderabad-500 007, Telangana State, India.
laboratory, Department 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-ob lanceolate 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.](image)

### 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 (Heidolph®LABAROTA EfficientAvaporators-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-M20A diode array detector (PDA), an SIL-20AC auto sampler, a DGU-20A, 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 Qualsil BDS C18 Column (250 x 4.6mm id; 5 µm, made in USA) were used. Chromatographic separation was achieved on LCGC Qualsil BDS C-18 Column using a mobile phase mixture of MEOH: H₂O in the ratio of 70:30 (v/v) and degassed using a vacuum degasser before use. The flow rate was set at 1 ml/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: H₂O 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 (Rₜ). Rₜ of standard 2-hydroxy-4-methoxy benzaldehyde was 6.35 min whereas the same when eluted for ecotypes showed Rₜ 5.96 min (Type-1), 5.98 min (Type-2), 6.04 min (Type-3), 6.04 min (Type-4), 6.07 min (Type-5), 6.08 min (Type-6) and 6.13 min (Type-7). Sreelekhā 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

<table>
<thead>
<tr>
<th>Name</th>
<th>Hexane</th>
<th>Chloroform</th>
<th>Acetone</th>
<th>Methanol</th>
<th>Aqueous extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloids</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Saponins</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Carbohydrates</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Cardiac glycosides</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Proteins</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Triterpenoids</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Phenols</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Steroids</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Coumarins</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

+ = Present, - = Absent

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

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

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.
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 presence of alkaloids, flavonoids, steroids, triterpinoids, 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.

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Conflict of interest

We declare that we have no conflict of interest.

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