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### Identification and Estimation of Wedelolactone in *Coldenia procumbens* linn.

Beena. P<sup>1</sup>., Purnima. S<sup>2</sup>., Kokilavani. R<sup>3</sup>

<sup>1</sup>College of Pharmacy, Sri Ramakrishna Institute of Paramedical Sciences, Coimbatore, Tamil Nadu, India

<sup>2</sup>S. B College of Pharmacy, Sivakasi, Tamil Nadu, India

<sup>3</sup>Department of Biochemistry, Kongunadu Arts and Science College, Coimbatore, Tamilnadu, India

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#### ABSTRACT

The basic objective of this work was to identify and estimate the active constituents present in the herb *Coldenia procumbens* Linn. A survey of literature showed that the plant has not been exploited properly and remains a silent drug in herbal medicine. In this work, the coumestan derivative wedelolactone was identified in the methanolic extract of the plant by TLC. The concentration of wedelolactone in the plant extract was found to be 2.2%w/w. An HPLC method was developed for the estimation and the developed HPLC method was validated with respect to LOD, LOQ and linearity. This is the first such report about the plant.

**Key words:** *Coldenia procumbens* Linn, wedelolactone, TLC, HPLC.

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#### INTRODUCTION

*Coldenia procumbens* Linn (Family: Boraginaceae) is a procumbent, deep rooted hairy herb found as a weed in moist places. It is found throughout in India, Srilanka and in other tropical countries. It is used for the suppuration of boils, fever, piles and scorpion sting. In spite of all these uses, the plant has not been evaluated chemically and biologically. [1, 2]

Wedelolactone is a coumestan derivative found in *Eclipta alba* and *Wedelia calendulaceae* Less. Wedelolactone is a potent and selective 5-lipoxygenase inhibitor. Wedelolactone and demethyl wedelolactone exhibit antihepatotoxic activities in carbon tetra chloride, galactosamine hydrochloride and phalloidin induced liver damages in rats. [3, 4]

The aim of the work was to identify and estimate the principal chemical constituent present in the methanolic extract of *Coldenia procumbens* Linn.

## MATERIALS AND METHODS:

### Plant source:

Leaves of *Coldenia procumbens* Linn were collected from west Tambaram, Chennai, India and were authenticated by Prof. P. Jayaraman of Plant Anatomy Research Centre (PARC), Chennai. These were freed from earthy material, washed, shade dried and powdered.

### Materials

- 1) Wedelolactone was obtained as gift sample from Laila Impex, Vijayawada, India.
- 2) Precoated silica gel G plates were obtained from Merck.
- 3) Shimadzu LC 2010 A HPLC system with following configurations.

Low pressure gradient quaternary pump, Auto injector, Multi wavelength UV array detector, Column oven and degasser, Class-V P 6.01 data station.

### Extraction

One Kg of powdered ariel parts of *Coldenia procumbens* Linn was taken and 2500 ml of 95% methanol was added. It was refluxed for 2 hours and filtered through muslin cloth while hot. Filtrate was concentrated, evaporated and standardised.

### Identification of active constituent by TLC:

1 mg/ml of wedelolactone in methanol was used as standard solution. 200 mg extract of *Coldenia procumbens* Linn was dissolved in 6 ml methanol by gentle heating, filtered through whatmann filter paper and volume was made upto 10 ml with methanol. Precoated silica gel G plates were used as the stationary phase. Mobile phase used was Toluene: Ethyl Acetate: Formic acid (5:4:1). The standard and plant extract solutions were applied on to the plates as 6 mm bands by means of an applicator. After development, the plates were dried and sprayed with Vannilin- Sulphuric acid heated to 110° C for 10 minutes and observed under daylight.

### Estimation of wedelolactone by HPLC:[5-7]

The stationary phase used was Lichrocart C<sub>18</sub> and mobile phase was water: acetonitrile (65:35 v/v). 100 µg/ml of wedelolactone in methanol was prepared. From this working standard solutions of 100-500 ng/ml were prepared in mobile phase. 1 mg of plant extract was dissolved in 50 ml methanol and vortexed to get clear solutions and made up to 200 ml with mobile phase. Detection was done at 254 nm. The flow rate was 1 ml/min and 50µl volume of samples were injected. The developed HPLC method was validated with respect to LOD, LOQ and linearity.

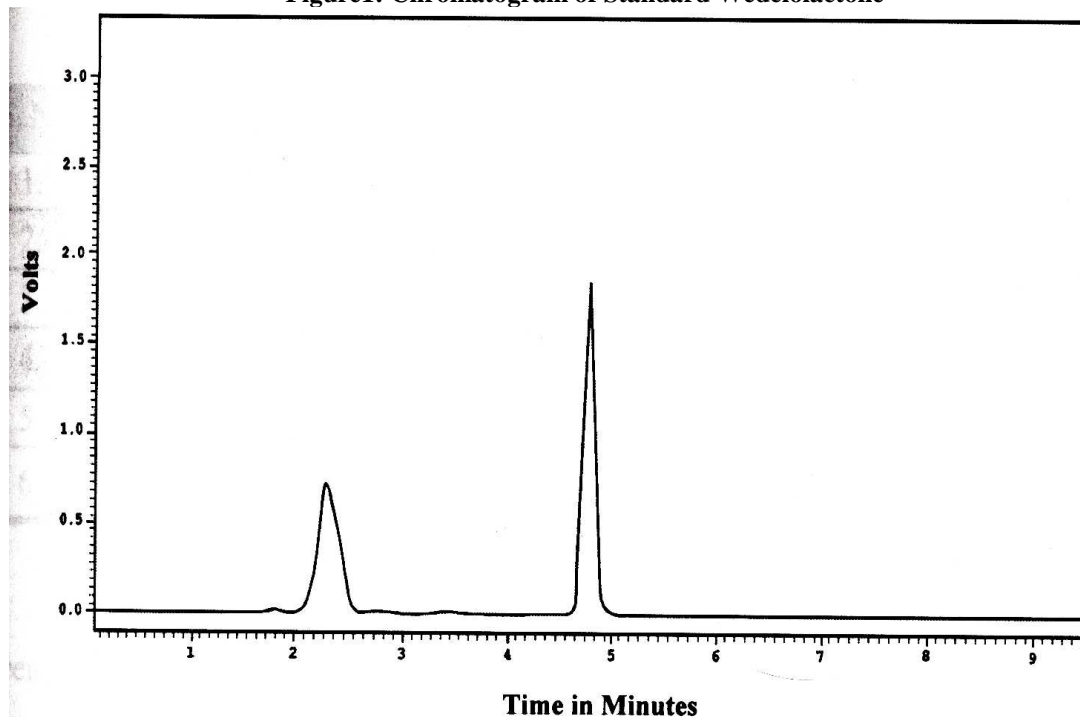
## RESULTS AND DISCUSSION

Identification of wedelolactone in *Coldenia procumbens* Linn was done by TLC and the results were tabulated (Table 1).

TABLE1: TLC OF *Coldenia procumbens* Linn

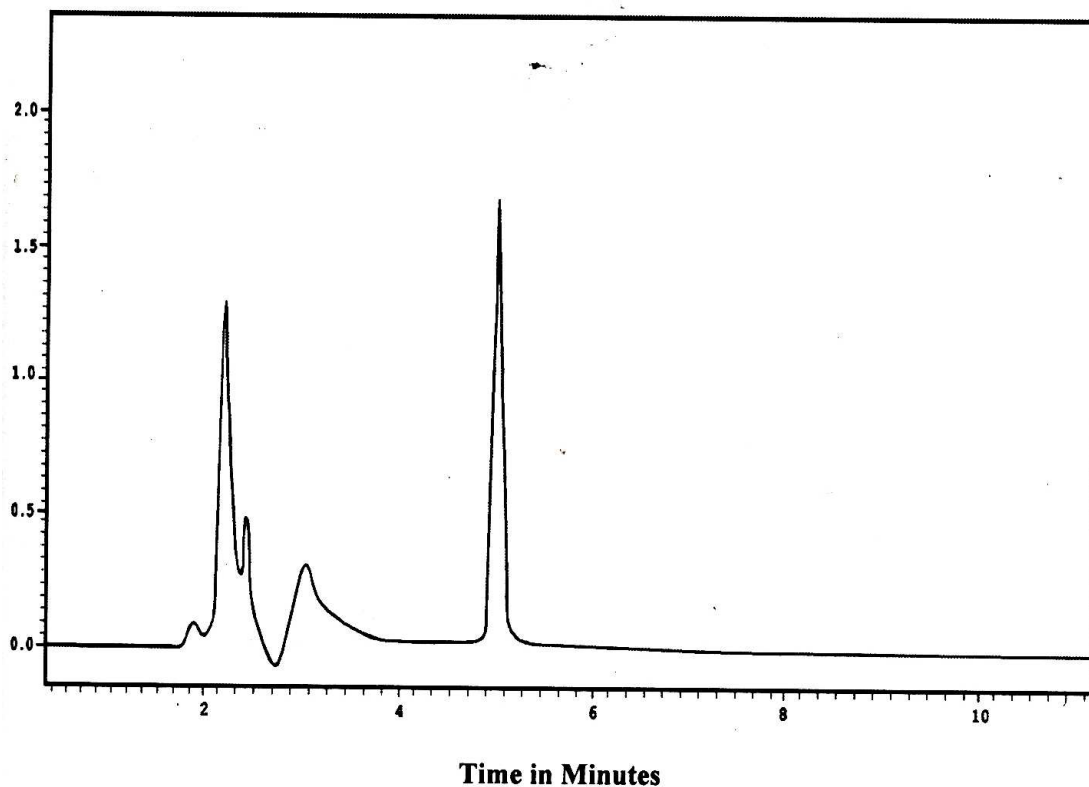
Extract	No. of spots	Spot no. and colour	R <sub>f</sub> value
<i>Coldenia procumbens</i> Linn	4	I-Blue	0.50
		II- Pink	0.77
		III -Violet	0.88
		IV - Green	0.92
Wedelolactone	1	Green	0.90

Figure1: Chromatogram of Standard Wedelolactone



Drug	Retention time	Area	Concentration
Wedelolactone	4.75 min	4011014	100 ng/ml

Figure 2: Chromatogram of *Coldenia Procumbens* Linn



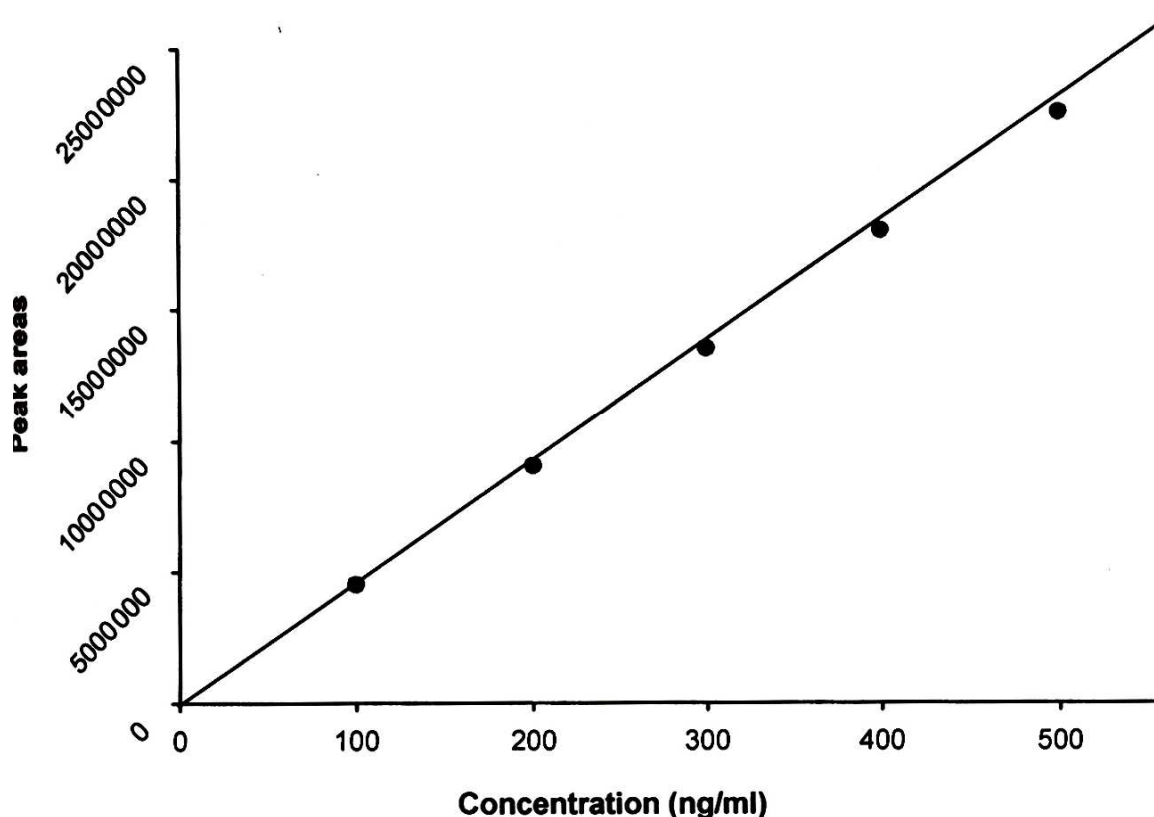
Drug	Retention time	Area	Concentration
Wedelolactone	4.99 min	5004518	110.94 ng/ml

HPLC was performed using optimized chromatographic conditions. The chromatogram of standard wedelolactone and *Coldenia procumbens* Linn is given in Figure 1 & 2.

An external standard is used for quantification. Peak identification was done based on retention times, comparison with standards and co-chromatography with standards. The chromatogram of *Coldenia procumbens* Linn extract showed a peak corresponding to that of pure wedelolactone. Various concentrations of standard wedelolactone were injected and their peak areas were recorded.

A calibration curve was drawn by plotting peak area versus concentration of standard (Figure3). The peak area of wedelolactone in the plant extract was also recorded. From the standard graph, the percentage of active constituent in the plant extract was calculated and found to be 2.2% w/w.

Figure3: Calibration Curve for Wedelolactone



Due to natural origin and complex structure, some peculiarities regarding the assessment of quality, safety and efficacy has to be considered in case of any herbal extract. Hence, the source of raw material is very important in case of herbal extracts because the quality and quantity of the active ingredient may vary due to different cultivation and harvesting methods. The raw materials derived from different sources differ in the percentage of active constituents. Hence the exact amount of active principle should always be specified from the source of raw material.

*Eclipta alba* and *Wedelia calendulaceae* Less are some plants which have been proven to have hepatoprotective activities due to the presence of wedelolactone which, in turn has proven anti hepatotoxic activity. In conclusion, the identification of wedelolactone in *Coldenia procumbens* Linn and its quantification points to the potential hepatoprotective activity of the plant.

**Acknowledgement**

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