

# Estimation of Wedelolactone in *Eclipta alba* and *Wedelia chinensis* and Comparative Antimicrobial Activity against Pathogenic Bacteria

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**Abstract-** *Eclipta alba* (L.) Hassk. and *Wedelia chinensis* (Osbeck) Merr., both belonging to the Asteraceae family, are well known in traditional medicine. Wedelo-lactone, a coumestan derivative, is considered a key bioactive constituent due to its potent anti-inflammatory, hepato-protective, analgesic, antibacterial and antidiabetic properties. The present study aimed to estimate wedelolactone content and evaluate the comparative antimicrobial activity in both plant extracts. HPLC and HPTLC methods were developed for evaluation of wedelolactone in both the plants *Eclipta alba* and *Wedelia chinensis*. Furthermore, the study investigated the in vitro antimicrobial activity of the crude extract of both plant against pathogenic bacteria, (*Staphylococcus aureus*, *E.coli*, *Pseudomonas aeruginosa* and a pathogenic yeast *Candida albicans*). A comparative study of the wedelolactone content and antimicrobial activity suggests a potential correlation between the concentration of this bioactive compound and its antimicrobial effects.

**Keyword:** *Eclipta alba*, *Wedelia chinensis*, HPLC, HPTLC, Antimicrobial activity, Anticandidal activity

## Introduction

Wedelolactone is a coumestan compound found in both *Eclipta alba* (Bhringraj) and *Wedelia chinensis* (Pilabhangra). It's a naturally occurring compound with various

pharmacological properties, including anticancer, anti-inflammatory, anti-diabetic, and anti-obesity effects, as well as protective effects on various organs. Both plants are recognized for their medicinal value, the concentration of bioactive compound like wedelolactone can vary in different parts of the plant.

*Eclipta alba* is an annual herb, has a short, flat or round stem, deep brown in color belonging to family Asteraceae. The plant is an active ingredient of many herbal formulations prescribed for liver ailments and shows effect on liver cell generation <sup>(1)</sup>. There are also reports of clinical improvement in the treatment of infective hepatitis <sup>(2)</sup>. *Eclipta alba* leaves showed antihyperglycemic activity <sup>(3)</sup>. The roots of *Eclipta alba* were found effective in wound healing <sup>(4)</sup>. In ayurvedic medicine, the leaf extract is considered a powerful liver tonic, rejuvenative, and especially good for the hair<sup>(5,6)</sup>. In Ayurveda, the plant is considered as a rasayana for longevity and rejuvenation. Dried leaves of *E. alba* are source of coumarins like wedelolactone and its derivative, dimethyl wedelolactone, isodemethylwedelolactone and strycolactone<sup>(7)</sup>.

*Wedelia chinensis* is a perennial herb belonging to family Asteraceae. The leaves are oval in form, succulent,<sup>(8)</sup> Flowers are 4-5 cm in diameter, yellow, tubular, and found in terminal or axillary heads. Various pharmacological

activities were found in *W. Chinensis* such as antioxidant, anti-inflammatory, analgesic, anti-cancer, antibacterial, antifungal, hepatoprotective and androgen suppressing activities<sup>(9)</sup>. The presence of Wedelolactone and dimethyl Wedelolactone in *Wedelia Chinensis* promote hair growth<sup>(10)</sup>.

Antimicrobial agents are essentially important in reducing the global burden of infectious diseases<sup>(11)</sup>. A vast number of medicinal plants have been recognized as valuable resources of natural antimicrobial compounds as an alternative that can potentially be effective in the treatment of these problematic bacterial infections<sup>(12)</sup>. According to the World Health Organization (WHO), medicinal plants would be the best source to obtain a variety of drugs<sup>(13)</sup>. The investigation into the antimicrobial activity of *Eclipta alba* and *Wedelia chinensis* against pathogenic bacteria is thus crucial for validating their traditional uses and exploring their potential as sources for developing new, effective and less resistance – prone antimicrobial therapeutics. The antimicrobial efficacy is particularly relevant in combating common bacterial pathogens, including both gram positive such as *Staphylococcus aureus* and Gram negative bacteria like *Escherichia coli*, *Pseudomonas aeruginosa* and fungi (*candida albicans*) which are frequently implicated in human infection.

This study will investigate the wedelolactone content in *Eclipta aba* and *Wedelia chinensis* plants and their antimicrobial activity against various pathogens. It aims to validate traditional uses of these plants and explore their potential as antimicrobial agents by quantifying wedelolactone and assessing their antibacterial and anticandidal effects.

## Material and Methods

### Collection and Authentication

The plants *Eclipta alba* and *Wedelia chinensis*, were collected from herbal garden of

Himalaya wellness company, Dehradun, UK, India and were identify by the Department of Pharmacognosy, Himalaya wellness Company, Dehradun, UK.

### Sample Preparation for HPLC

5g of the coarsely powdered *Eclipta alba* and *Wedelia chinensis* leaves were refluxed under examination with 30 ml of methanol on a water bath for 30 minutes, cooled and filtered respectively. The residue refluxed further with methanol till the last extract turned colorless. It was cooled down and filtered. All the filtrates were combined and concentrated to 100 ml<sup>(14)</sup>.

**Standard preparation-** 0.01% w/v solution of wedelolactone was added in methanol. This was our Standard solution.

**Mobile phase Preparation-** 65 volumes of 0.1% v/v phosphoric acid were prepared by diluting 1 ml of phosphoric acid to 1000ml with water. These 65 volumes of 0.1% v/v phosphoric acid was mixed with 35 volumes of acetonitrile.

### HPLC Conditions

**Column-** C18 Shemadzu column 250= 4.6mm particle size 5 $\mu$ .

**Flow rate** – 1ml per minute

**Detection-** at 351 nm

**Volume of injection-** 20  $\mu$ l of sample and standard solution.

### Method of Analysis

Stabilize the instrument with the mobile phase till the baseline is satisfactory. Inject the standard solution and record the chromatogram. The % RSD between the results should be less than 2%. Similarly inject the sample solutions in duplicate and record the chromatogram.

### Calculation

$$\frac{\text{Area of sample}}{\text{Area of Standard}} \times \frac{\text{Area of sample}}{\text{Area of Standard}} \times \text{Purity}$$

## HPTLC Procedure

### Material and Reagents

**HPTLC Plates-** Precoated silica gel 60F254 plates E.Merck.

**Wedelolactone Standard-** Accurately weigh a suitable amount of wedelo-lactone standard and dissolve it in methanol to prepare a standard solution (1mg/ml).

**Sample preparation-** Extract the plant material using an appropriate solvent (e.g., methanol, hexane and aqueous). Concentrate the extract for further analysis like HPTLC and antimicrobial activity.

**HPTLC instrumentation-** Applicator (e.g., Camag; Linomat), developing chamber (twin through glass chamber) **and documentation system.**

**Mobile phase-** The solvent system used was toluene: acetone: formic acid (11:6:1 v/v) for developing the chromate-gram<sup>(15)</sup>.

### Antimicrobial Activity

In the antimicrobial activity, the microorganisms used for the study are:

1. *Escherichia coli* - ATCC 8739
2. *Pseudomonas aeruginosa* – ATCC 9027
3. *Staphylococcus aureus* - ATCC 6538

## 4. *Candida albicans*. - ATCC 10231

These cultures were maintained on nutrient agar slants at first being incubated at 37°C for about 18-24 hours and then stored at 4°C as stock for antimicrobial activity. Fresh culture was obtained by transferring a loop full of cultures into nutrient broth and then incubated that 37°C overnight. To test antimicrobial activity, the well diffusion method was used.

The agar well diffusion method<sup>(16)</sup>, is used to determine the antimicrobial activity of plant extracts. This method involves creating wells in an agar plate inoculated with a microorganism, filling these wells with plant extracts, and observing the formation of zones of inhibition (clear areas) around the wells, indicating the presence of antimicrobial compounds.

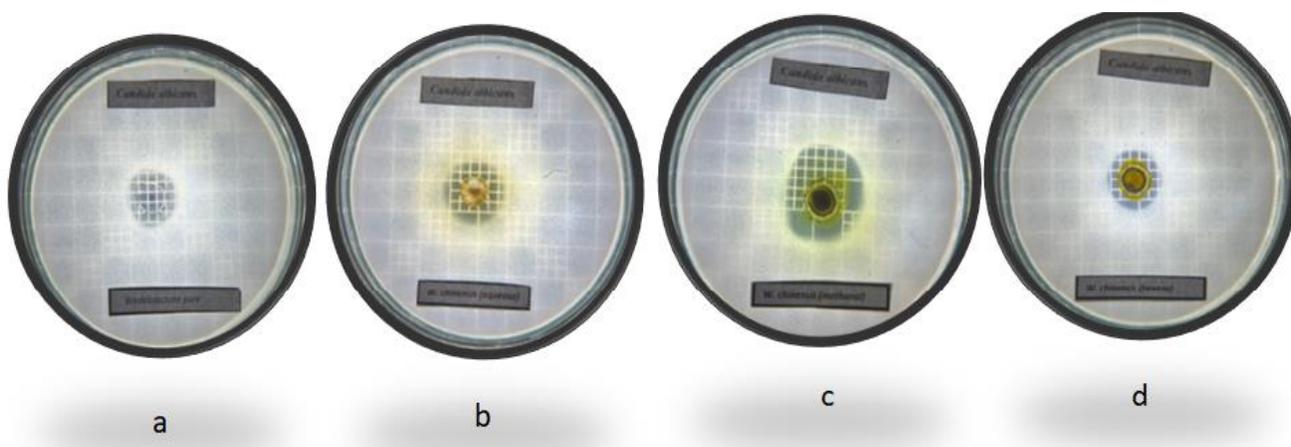
## Results and Discussion

Antimicrobial activity for different extracts of *Wedelia chinensis* and *Eclipta alba*, along with wedelolactone tested against four microorganism: *S.aureus* (Gram – positive bacteria) *E.coli* and *P.aeruginosa* (Gram – negative bacteria) and *C.albicans* (Pathogenic Yeast).

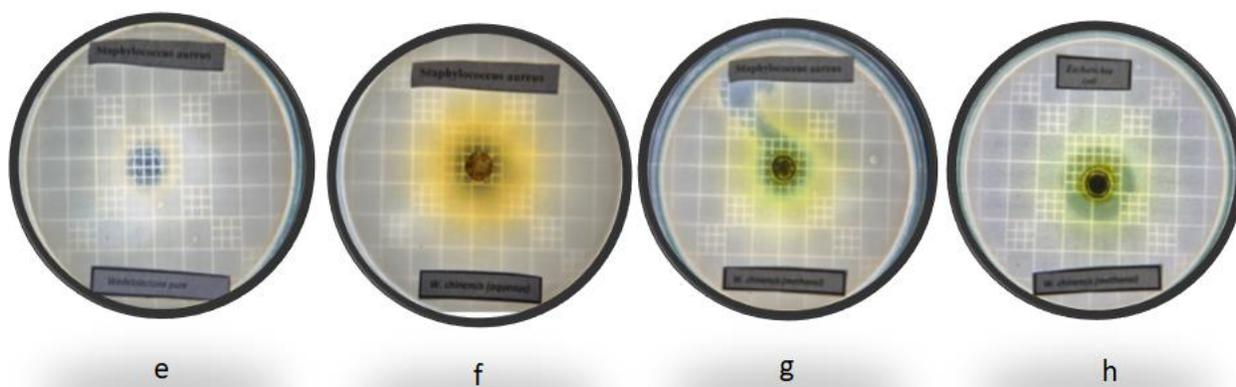
**Table-1 Antimicrobial activity of *Wedelia chinensis*, *Eclipta alba* and wedelolactone.**

Microorganism	Wedelia chinensis in( mm)			Eclipta alba in( mm)			Wedelolactone ( 1mg/ml)	Positive control
	ME	HE	AE	ME	HE	AE		
<i>S. aureus</i>	16	11	13	ND	ND	ND	14	36
<i>E. coli</i>	18	13	12	17	10	ND	15	37
<i>P. aeruginosa</i>	10	13	12	10	ND	ND	ND	39
<i>C. albicans</i>	25	17	18	17	20	ND	17	35

**Note:** ME- Methanol extract; HE- Hexane extract; AE- Aqueous extract; ND-Not Detected



**Figure-1 Anticandidal activity against *Candida albicans* of**  
**a) Wedelolactone b) HE of *W.chinensis* c) ME of *W.chinensis* d) AE of *W.chinensis***



**Figure- 2 Antibacterial activity of *Wedelia chinensis***  
**e) Wedelolactone against *S.aureus* f) AE against *S.aureus* g) ME against *S.aureus* h) ME against *E.coli*.**

Different extracts of both plants were evaluated for their antimicrobial potential against microorganisms. In this study using well agar method (Table-1) summarizes and revealed the results obtained and listed the only plant species that presented some activity against at least one microorganism. Both plants showed antimicrobial activity. *E. alba* and *Wedelia chinensis* are the best drug for treating liver cirrhosis and infective hepatitis; wedelolactone is considered as the active principles responsible for the use of these drugs in liver disorders <sup>(17)</sup>.

The varying activity across different extracts (methanol, hexane, aqueous) highlights the importance of extraction solvent. Methanol

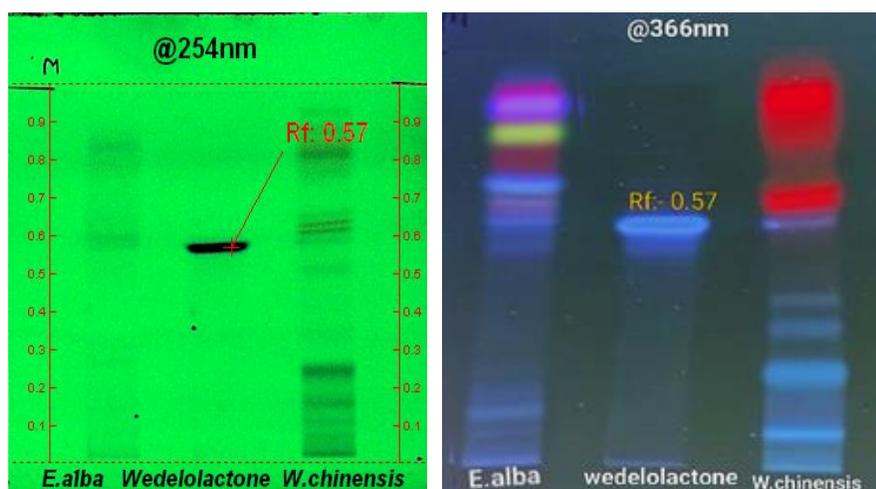
extract generally appear to be more effective, suggesting that the active antimicrobial compound in these plants are more soluble in methanol. The lack of activity from *E.alba* aqueous extract is particularly notable and indicates that its active components might not be water soluble.

*C.albicans* seems to be particularly more susceptible to the *Wedelia chinensis* showing zone of inhibition of (25mm) and *Eclipta alba* showing zone of inhibition of (20 mm) respectively.

The HPTLC analysis was performed on *Eclipta alba* and *Wedilia chinensis*, along a standard of wedelolactone, using two detection wavelength at 254nm and 366nm.

At 254nm a dark band corresponding to the wedelolactone standard was observed at an Rf value of 0.57. In *E.alba* a prominent band was observed at Rf 0.57. This suggests the presence of a compound in *E.alba* with similar UV absorption to wedelolactone. The *W.chinensis* also showed multiple bands, a compound was observed at 0.57 Rf consistent with that of wedelolactone, indicating the presence of this compound in *W.chinensis* as well.

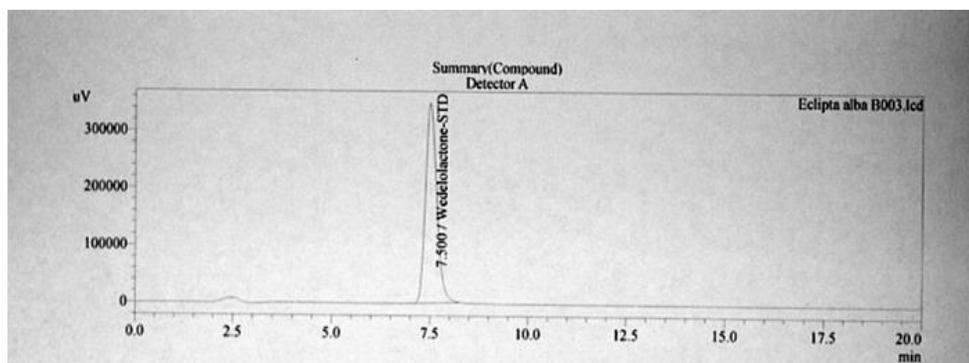
Under 366nm (fluorescence), the wedelolactone standard shows a distinct blue fluorescence at Rf of 0.57. The *E. alba* and *W. chinensis* display a strong fluorescent band at same Rf value identical to wedelolactone. Many fluorescent bands were also visible in both plant extracts, indicating a diverse array of different compounds.



**Figure-3 HPTLC Chromatogram of *E. alba*, *W. chinensis* and Wedelolactone**

In HPLC, the chromatogram shows a prominent peak at Rt (Retention time ) of 7.487 minutes for standard wedelolactone. The sharp and symmetrical nature of the wedelolactone peak indicates good chromatographic separation and efficient column performance this suggests the

HPLC methods developed or utilize is suitable for analyzing wedelolactone. The data confirm the presence of wedelolactone in the *Eclipta alba* and *Wedelia chinensis* with a retention time of 7.522 and 7.429 minutes respectively.



**Figure-4 HPLC Chromatogram of wedelolactone standard t Rt-7.487**

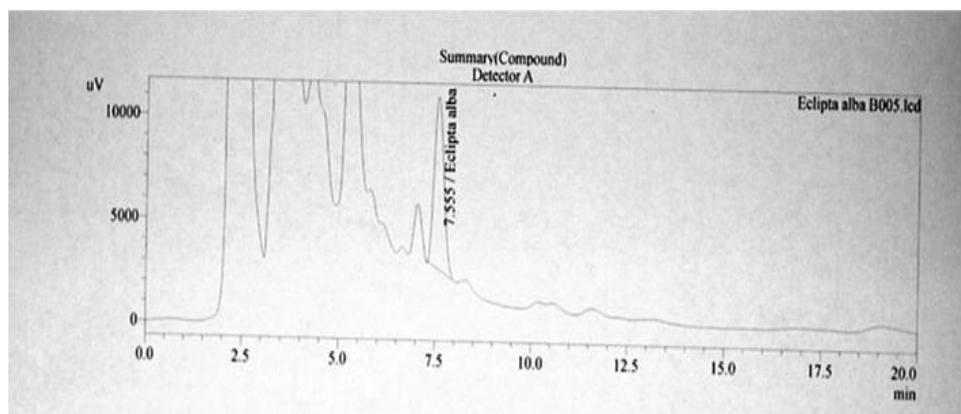


Figure-5 HPLC Chromatogram of *Eclipta alba* Rt-7.522

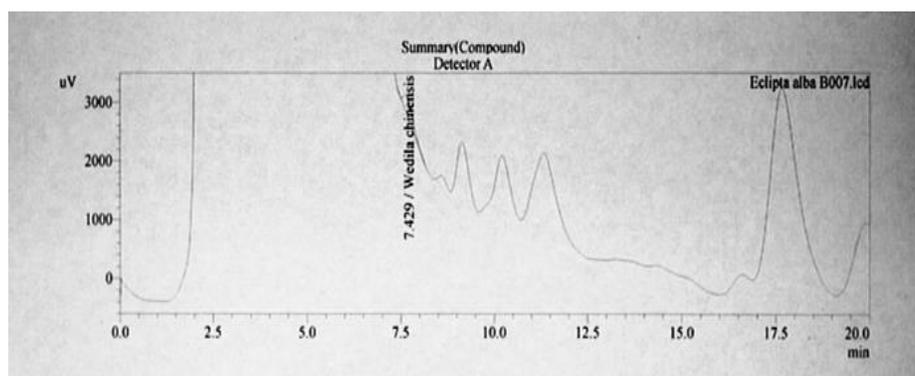


Figure-6 HPLC Chromatogram of *Wedelia chinensis* Rt-7.487

Table-2 Calculation of Wedelolactone in different plant

Sr. No.	Rt (retention time)	Plant Name	Plant part used	Wedelolactone (% w/w)
1	7.522	<i>E.alba</i>	Whole plant	0.040%
2	7.429	<i>Wedelia chinensis</i>	Whole plant	0.031%

*E.alba* contains 0.040% (w/w) of Wedelolactone, which is substantially higher than the 0.031 % (w/w) found in *Wedelia chinensis*. This suggests that *E.alba* is a richer natural source of wedelolactone compared to *Wedelia chinensis* under the conditions of this analysis and using the whole plant.

## Conclusion

In the present study we found Wedelolactone content is higher in *Eclipta alba* compared to *Wedelia chinensis*. Wedelolactone is a known bioactive compound with various medicinal properties. This difference in concentration makes *Eclipta alba* the more commercially viable and efficient plant source for the

extraction and isolation of wedelolactone. The superior antimicrobial activity of *Wedelia chinensis* likely stems from the synergistic effects of other phytochemicals present in its extract revealed the complexity of natural product, where the collective action of multiple compounds often dictates biological activity rather than the concentration of a single marker compound. Methanol is more suitable solvent for extraction.

## Disclaimer Statement

Authors declare that no competing interest exists. The products used for this research are commonly used products in research. There is no conflict of interest between authors and pro-

ducers of the product.

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