# In vitro Antibacterial Activity of Wedelolactone from *Eclipta alba* Against Human Pathogenic Bacteria

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**Abstract**

Aerial parts of *Eclipta alba* are used traditionally for the treatment of several diseases of liver, skin and stomach. Methanolic extract and active principle compound of Iraq herb, *Eclipta alba* was tested for in vitro antimicrobial studies. It was evaluated using zone of inhibition studies and minimum inhibitory concentration was observed at the concentration of (0.08,0.04,0.02,0.01) g/ml against pathogenic bacteria. Phytochemical screening of the extract revealed the presence of active principles, coumestans (Wedelolactone), Alkaloids, Flavonoid, Glycosides, Triterpenoids, Tannins and Saponins. Methanolic extract fraction (wedelolactone) showed enhanced antimicrobial activity. *Staphylococcus aureus, Pseudomonas aeruginosa, Klebsiella pneumonia* and *Acinetobacter ssp.* were most pathogen and resistant bacterial strain. These results suggest coumestans/wedelolactone as a promising antimicrobial agent.

***Keywords****: Eclipta alba , wedelolactone , antimicrobial activity .*

## فعالية المضاد البكتيري الـWedelolactone المعزول من نبات *Eclipta alba* ضد بعض البكتريا الممرضه للانسان

**م.م. زينب عباس شنشول**

**الخلاصة**

تستخدم الاجزاء الهوائيه لنبات *Eclipta alba* لعلاج العديد من امراض الكبد والجلد والمعدة تم اختبار المستخلص الميثانولي (المركب الفعال) للعشب العراقي . الدراسات المختبرية اظهرت فعالية لنبات *Eclipta alba* ضد البكتريا من خلال مناطق التثبيط والتثبيط الادنى لتراكيز المختلفه(0.08,0.04,0.02,0.01)للمادة .الفعالة الـWedelolactone بينت له فعالية ضد البكتريا الممرضة. لما يحتويه المستخلص الميثانولي لماده الـWedelolactone من صنف الكومارينات على مركبات اخرى ساندة مثل القلويدات والفلافونات وكلايكوسيدات وتانينات وصبونات .. الخ اظهرت فعاليه تثبيطية ضد سلالات *Staphylococcus aureus , Pseudomonas aeruginosa, Klebsiella pneumonia* and *Acinetobacter ssp.* وكانت اغلبها سلالات ممرضه ومقاومه للادوية وبالنتيجه بينت فعاليه الـWedelolactone في تثبيطها.

***الكلمات المفتاحية*** *: نبات العـرنس ، مادةالفعالة wedlelactone ، وفعالية المضاد البكتيري .*

## Introduction

Medicinal plants are important substances for the study of their traditional uses through the verification of pharmacological effects and can be natural composite sources that act as new anti-infectious agents. In order to find out new sources of drugs, a number of plants have been screened for wide range of biological activities. About 3,000 materials from 2,764 plant species have been screened for their pharmacological and chemotherapeutic properties [1]. *Eclipta alba* is a weed /herb growing in damp, moist puddles distributed in the tropical and subtropical regions of the world. India, in particular has yielded an incredible array of plant products that have drawn the attention of ethno pharmacologists from around the world. Traditionally used medicinal plants produce a variety of compounds of known therapeutic properties [2- 4]. Various biological activities are possessed by *E.alba*, such as memory disorders treatment, general tonic, edema, fevers and rheumatic joint pains treatment, digestion, hepatitis, enlarged spleen, antioxidant activity and skin disorders [5-7].

Wedelolactone is active principle compound of this liver disorder treating drug [8]. antibacterial [9]. it can be hypothesized that plants which survive in media rich in microbes most likely be possessing antimicrobial principles. However, up to date, research has been done to investigate various pharmacological activities and antimicrobial activity of only crude extracts of this traditionally used herb. We report here our findings on antibacterial effects of wedelolactone (Fig. 1), the principle active compound, extracted from *E. alba*.

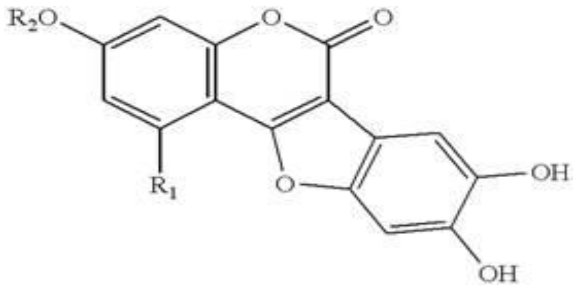



**Figure (1):** Chemical structure of Wedelolactone (R1-OH, R2-CH3)

Main active principles consist of coumestans like wedelolactone, desmethylwedelolactone [11], furanocoumarins, oleanane & taraxastane glycosides [12]. Various biological activities are possessed by *E. alba*, such as memory disorders treatment, general tonic, edema, fevers and rheumatic joint pains treatment, digestion, hepatitis, enlarged spleen, antioxidant activity and skin disorders [13-15]. Wedelolactone is active principle compound of liver disorder treating drug [16].

It also exhibits Trypsin inhibitory effect [17, 18]. Treatment of cirrhosis of the liver and infectious hepatitis. However, up to date, research has been done to investigate various pharmacological activities and antimicrobial activity of only crude extracts of this traditionally used herb [19].

## Materials and methods

### Plant collection

The aerial parts of *Eclipta alba* (Asteraceae) were collected locally from different places nears the Tigris river in Bagdad, during November 2011 to January 2012. The collected plants were cleaned with distilled water [19].

### Qualitative estimation of primary and secondary plant metabolites

All estimations were done following [2,4]. Different aerial parts of the plant were dried at room temperature, and extraction of crude wedelolactone by soxhlet thumble was filled with twenty grams of dried plant powder, and put in the soxhlet apparatus solvent which was tested (Methanol 70% v/v) at 50°C for 36 hours [20]. Filtration and concentration the extracted sample, the extracted sample were filtered through filter paper (what man no.1) the filtered sample was concentrated by using rotary evaporator at a temperature varies according to the solvent which was used then the yields were kept in container at 4°C until further use.

### Isolation of Wedelolactone

The powder was subjected to fractionation by column chromatography on silica gel, eluted with the solvent of increased polarity i.e. Non-polar - polar - highly polar. The coumestans are polar compounds so the solvent combination found suitable for their elution was HPLC mobile phase acetonitrile–water (40:70) was prepared at 351 nm according to [21].
according to the following equation:

$$\text{The concentration \%} = \frac{\text{AUC (standard)}}{\text{AUC (test)}} \times 100$$

AUC =Area under Curve [20].

**Preparation of samples(Wedelolactone) for testing**

A series of wedelolactone concentration dissolved with methanol were prepared as followed (0.08, 0.04, 0.02, 0.01) g/ml.

**Microorganisms (Sensitivity test for purified wedelolactone extract):**

Brain heart infusion agar (BHIA) was prepared according to the production company; the sterilized (BHIA) was seeded with one of the following tested (*Staphylococcus aureus, Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Acinetobacter spp*.) according to McFarland 0.5CFU/ml. After solidification, the wells were done, then 100 µl of different wedelolactone concentration were put in the wells, for all types of bacterial isolate used., the Petri dishes were incubated at 37 °C for 18 hours [23].

**Result and discussion**

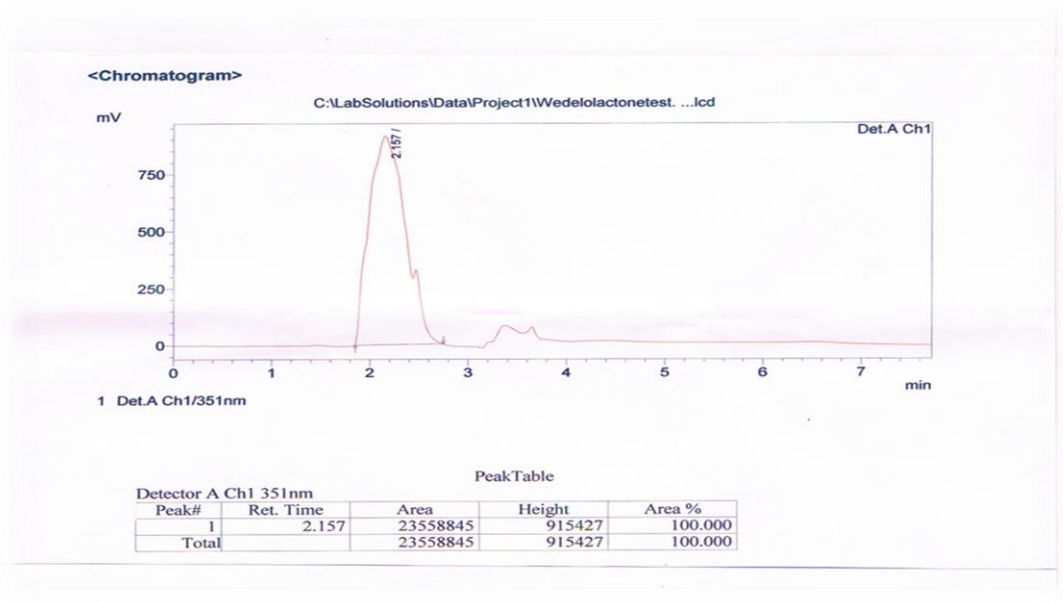
The result of wet quantity measurement for the aerial parts of plant and extraction method by using solvent (Diluted Methanol 70% v/v ) reached 76% The characterization of phytochemical compound of the crud extract showed there were different compounds (Coumarines , Flavones ,Volatile Oil ,Tannins , Saponines , Glycosides ,Carbohydrates ,Alkaloids , Resins) But in different percentage and this result agreed with [21] The results of the presence of various primary and secondary metabolites in methanol extract (Table 1) reported negative for antraquinones throughout herb.

**Table (1):** Quantitative estimation of the various primary and secondary metabolites

| No. | Chemical Test | Natural plant extract | | |
|---|---|---|---|---|
| | | **Leaf** | **Stem** | **Root** |
| 1 | Alkaloids | + | + | - |
| 2 | Coumestans | + | + | + |
| 3 | Anthraquinones | - | - | - |
| 4 | Phenolics | + | + | + |
| 5 | Saponins | + | + | + |
| 6 | Steroids | + | + | + |
| 7 | Proteins | + | + | + |
| 8 | Amino-acids | + | + | + |
| 9 | Reducing sugar | + | + | - |
| 10 | Flavonoids | + | + | + |

**Isolation of Wedelolactone by using the High Performance Liquid Chromatography (HPLC) technique**

For certification of the previous characterization and for determination the degree of wedelolactone sample partial purity and comparison with standard one this analysis was done. The result of this test showed there was close compatible curves for the sample with that of standard one in the shape, but slightly different in the retention time, that where the peaks were appeared. The partial purified wedelolactone sample appeared at (2.157) minutes as shown in Figure (2) while standard wedelolactone at (2.163) minutes as shown in Figure (3), from these results, the concentration ratio of wedelolactone tested sample was calculated, which was reached 99.72%, and these results indicated the high purity of the tested sample. And these results agreed with [20,22]




PeakTable

Detector A Ch1 351nm

| Peak# | Ret. Time | Area | Height | Area % |
|---|---|---|---|---|
| 1 | 2.157 | 23558845 | 915427 | 100.000 |
| Total | | 23558845 | 915427 | 100.000 |

**Figure (2):** The wedelolactone in the High Performance Liquid Chromatography (HPLC) technique

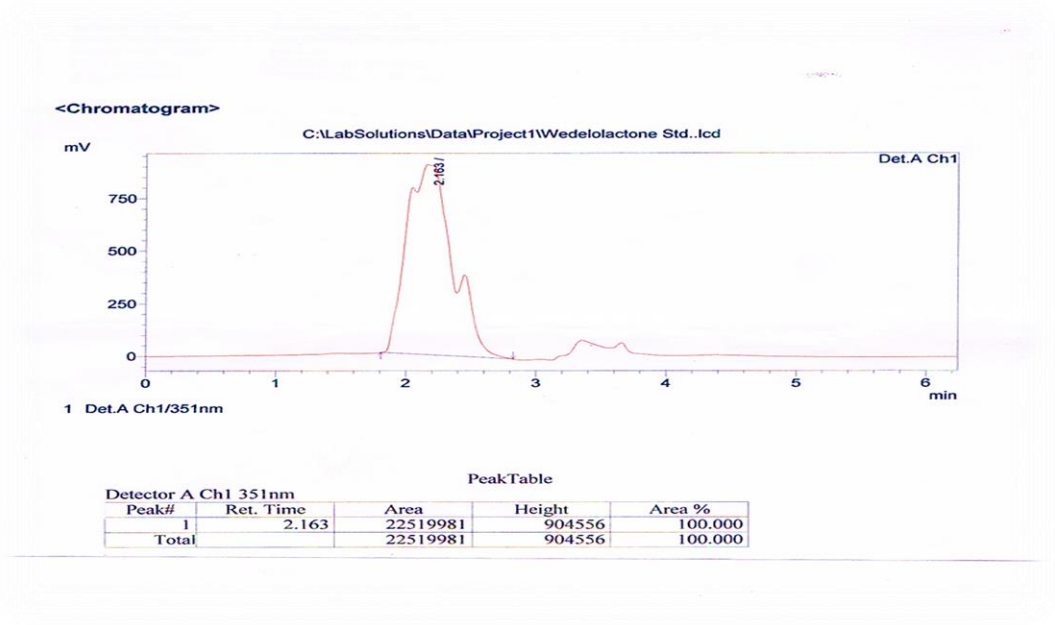



**Figure (3):** The wedelolactone standard sample curve using the High Performance Liquid Chromatography (HPLC) technique at absorbance 351 nm

**In vitro sensitivity test (MIC)**

The results of the antibacterial effect of the wedelolactone against several bacterial isolates by using serial concentrations showed there were mild inhibition effect for both types of bacterial isolate (positive and negative gram stain), these results were not confirmed with that reported by [23-26], the slipping of the result from the global reports may be due to the differentiation in the wedelolactone concentration which were u

sed.

**Table (3):** The MIC measurement of the wedelolactone by the well Diffusion method

| Bacteria | 0.01 | 0.02 | 0.04 | 0.08 | Solvent |
|---|---|---|---|---|---|
| ***staphylococcus aureus*** | 12mm | 12mm | 14mm | 18mm | 11mm |
| ***Pseudomonas aeruginosa*** | 11mm | 12mm | 13mm | 16mm | 12mm |
| ***Klebsiella pneumoniae*** | 12mm | 13mm | 13mm | 15mm | 12mm |
| ***Acinetobacter spp.*** | 11mm | 12mm | 13mm | 15mm | 11mm |

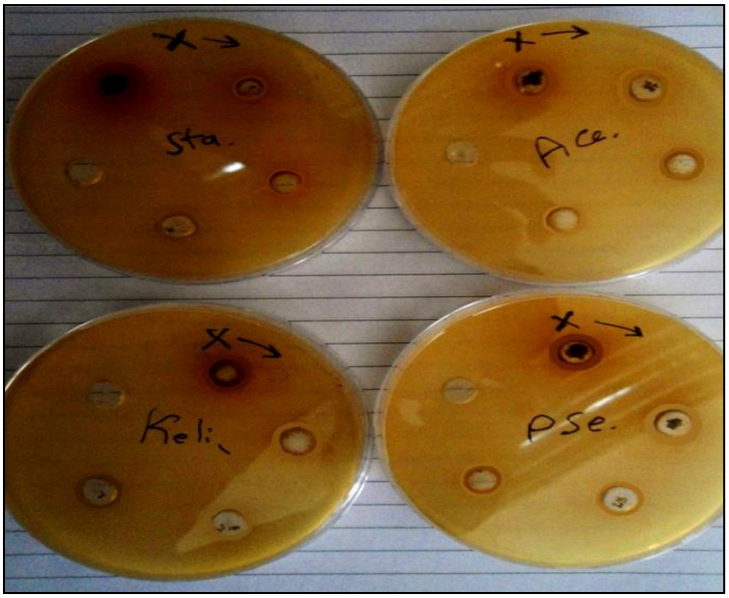



**Figure (4):** showed the MIC effect in the plate

The inhibitory effectiveness of wedelolactone may be due to it is belonging to a coumestan compound, because this type of coumestan is one of the lipophilic, simple molecular Aromatic structure, and simple [26].

## Conclusion

*Eclipta alba* is a weed; the most weight of the plant is water, which reached in to 90%. The best solvent which could extracted the active material was Methanol 70%.

The characterization of phytochemical compound in the crud extract showed there were (Coumarines , Flavones , Tannins , Saponines , Glycosides ,Carbohydrates ,Alkalaoids , Resins) But in different percentage. the HPLC detection for wedelolactone showed appositive result. The using of the wedelolactone internally showed effective and inhibition growth bacteria (gram negative and gram positive).

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